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Understanding activity patterns of aquatic animals through acoustic telemetry: application to the marine fish species *Epinephelus marginatus* (Lowe 1834)

Comprensión de los patrones de actividad de animales acuáticos a través de la telemetría acústica: aplicación a la especie de pez marino *Epinephelus marginatus* (Lowe 1834)

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Dissertation submitted by
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Un mero investigador

22 de marzo 2021

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Resumen

RESUMEN

El estudio del comportamiento animal es el centro de atención en multitud de investigaciones enmarcadas en distintas disciplinas científicas (evolución, ecología, psicología, medicina, economía, etc.). Sin embargo, el estudio del comportamiento animal es bastante complejo. Uno de los aspectos que dificultan el estudio del comportamiento animal está relacionado con las limitaciones tecnológicas a la hora de adquirir los datos, las cuales dependen del tipo de tecnología y suelen ser mayores en los entornos acuáticos que en los terrestres debido a las condiciones físico-químicas que impone el agua. Los métodos de recogida de datos basados en “archival tags”, por ejemplo, implican la necesidad de volver a capturar individuos después de un cierto tiempo. Esto implica, por tanto, un mayor esfuerzo de muestreo y la limitación de tener que utilizarlos con especies de fácil recaptura. Incluso podría ser necesario aumentar el tamaño de la muestra (número de individuos marcados) para tener en cuenta la probable pérdida de dispositivos. En cambio, los métodos de telemetría evitan la necesidad de volver a capturar individuos, ya que los datos se transmiten directamente a los receptores (por ejemplo, hidrófonos o satélites). Los dispositivos que transmiten datos se denominan transmisores, y suelen hacerlo de forma inalámbrica, principalmente mediante señales electromagnéticas o acústicas. Sin embargo, las tecnologías de telemetría tienen otras limitaciones. En primer lugar, son más caras que métodos como los “archival tags”, los censos visuales o la grabación de videos En segundo lugar, la adquisición de datos puede depender de la posición del animal con respecto a los receptores, lo que da lugar a lagunas de datos cuando, por ejemplo, los animales están ocultos y la señal no llega a los receptores. Una tercera limitación está relacionada con la duración de las baterías. Parte de la energía de las baterías de los transmisores se utiliza para enviar las señales, lo que reduce el tiempo de seguimiento de los individuos en comparación con otros métodos no telemétricos, como los “archival tags”. Otra limitación importante de las tecnologías de telemetría está relacionada con la capacidad de transmisión de datos. Algunos tipos de transmisores pueden enviar una cantidad limitada de información en cada señal, lo que repercute tanto en la calidad como en la cantidad de datos obtenidos. Un ejemplo es el uso de transmisores acústicos equipados con un sensor de

temperatura y otro de profundidad: estos dispositivos no pueden enviar datos de temperatura y profundidad simultáneamente, sino que deben enviarse por separado, lo que reduce el número de datos obtenidos de cada tipo. Por último, otra limitación de los métodos de telemetría es el tamaño de los transmisores. Algunos de ellos (por ejemplo, los transmisores por satélite) son relativamente grandes, lo que impide su uso en especies pequeñas.

La actividad, definida por algunos autores como "el movimiento general, o específico, de un animal", es uno de los temas más abordados en los estudios del comportamiento animal. La importancia de este tema y la amplitud de este término han dado lugar a miles de estudios en los que se ha evaluado la actividad de los animales de una u otra manera. Uno de los métodos más novedosos para inferir la actividad de los animales salvajes es el de los acelerómetros. Los acelerómetros son dispositivos que miden la aceleración de un cuerpo, normalmente en sus tres dimensiones espaciales (x, y, z), aunque la pueden medir en menos. Un acelerómetro triaxial perfectamente calibrado y estático sobre cualquier superficie mediría 9,8 $m\ s^{-2}$ entre los tres ejes. Cuando estos dispositivos se fijan al cuerpo de los animales, los valores de aceleración en los tres ejes ayudan a entender qué tipo de movimientos (es decir, comportamientos) realizan. Además, ha demostrado ser válido para inferir la actividad en numerosas especies, dada la estrecha relación entre la aceleración y el coste energético. Una cuestión que ha cobrado especial relevancia en los últimos años es el impacto del cambio inducido por el hombre en la actividad de los animales, tanto espacial como temporalmente. Algunos trabajos han mostrado cómo las actividades humanas están impulsando derivas temporales en los patrones de actividad de numerosas especies de mamíferos, que tienden a aumentar su nocturnidad como respuesta a las perturbaciones humanas. Además, se sabe que las actividades humanas afectan a las especies de forma diferente por múltiples razones (diferencias en la personalidad de los animales, la distribución temporal de las actividades humanas, etc.), y podrían causar impactos a nivel de comunidad al alterar la co-ocurrencia de las especies y sus interacciones. Un grupo de especies en el que la actividad humana afecta especialmente a los patrones de actividad es el de los depredadores, que suscitan un gran interés para los humanos y suelen ser más vulnerables a los cambios en la composición de la comunidad.

La telemetría acústica se ha convertido en una de las principales tecnologías para estudiar el comportamiento de los animales en el ámbito acuático, incluidos los patrones de actividad. Esto se debe a las buenas propiedades de propagación de las ondas acústicas en el medio acuático, lo que permite el fácil desarrollo de una red de transmisores y receptores. Por el contrario, la capacidad de propagación de las ondas electromagnéticas en el medio acuático es débil, lo que restringe el uso de transmisores satelitales a especies que viven en estrecho contacto con la superficie del agua. Un ejemplo claro de la utilidad de la telemetría acústica para estudiar el comportamiento de los animales acuáticos lo proporciona la especie de pez bentónica *Epinephelus marginatus* (Lowe 1834), comúnmente conocida como mero. Gracias a esta tecnología, se ha podido confirmar el marcado comportamiento sedentario de esta especie, que suele desplazarse dentro de áreas relativamente pequeñas. Esta información ha contribuido, por ejemplo, a reforzar los argumentos a favor de la creación de áreas marinas protegidas (AMP) aunque sean pequeñas, ya que podrían proteger los hábitats de esta especie de gran valor ecológico y socio-económico. Sin embargo, aún quedan por explorar muchos aspectos del comportamiento de esta emblemática especie, que podrían ser cruciales para su conservación. Un tema poco conocido, pero de especial relevancia, son los cambios de comportamiento inducidos por el hombre en los meros, especialmente los producidos por la actividad del buceo recreativo. El buceo recreativo es una actividad que ha crecido exponencialmente en la última década debido al creciente interés de la sociedad por el turismo de naturaleza. En este sentido, la telemetría acústica es una herramienta útil que puede ayudar a comprender dichos efectos. Sin embargo, en la actualidad, no hay ningún trabajo que conozcamos que evalúe el efecto del buceo en los patrones de actividad de los animales acuáticos mediante telemetría acústica.

A pesar de la utilidad de la telemetría acústica para evaluar la actividad de los animales acuáticos, tiene una serie de limitaciones técnicas que es importante tener en cuenta tanto a la hora de diseñar los estudios como de interpretar los datos. En primer lugar, los transmisores acústicos suelen colocarse en el interior del cuerpo del animal (por ejemplo, en la cavidad celómica) para evitar el biofouling y su posible pérdida por desprendimiento cuando funcionan durante largos periodos de tiempo (~ meses). Esto implica que los transmisores se quedan con el movimiento libre ya que no pueden fijarse dentro del cuerpo del animal, lo que aumenta la incertidumbre sobre el efecto que esto puede tener a la hora de comparar datos entre individuos. Otra preocupación que surge debido a las limitaciones de la telemetría acústica es la existencia de problemas de sesgo de muestreo. La variación en el comportamiento de los animales suele explicarse, al menos en parte, por el tipo de hábitat en el que se encuentran. En este sentido, es probable que la intensidad de la actividad animal también cambie entre hábitats. Esto puede suponer un problema a la hora de utilizar la telemetría acústica, ya que su rendimiento depende en gran medida de factores abióticos como el tipo de hábitat (asociado a la presencia de barreras a la propagación del sonido). Por lo tanto, la probabilidad de detectar las señales acústicas de los transmisores puede variar en función del hábitat en el que esté presente el individuo, lo que da lugar a un sesgo de muestreo. Si, por ejemplo, los individuos fueran más detectables en los hábitats en los que son más activos, obtendríamos una mayor proporción de valores de actividad altos respecto a la real. Otra duda surge del carácter intermitente de los datos. En ocasiones, es necesario o aconsejable promediar los valores de actividad brutos a lo largo de grandes intervalos de tiempo (por ejemplo, 15 minutos, 30 minutos, etc.). Sin embargo, con tamaños de intervalo de tiempo cada vez mayores, existe un mayor riesgo de muestrear la actividad solo durante una pequeña proporción de tiempo respecto al tiempo total, lo que plantea la incertidumbre sobre las consecuencias de utilizar diferentes tamaños de intervalo de tiempo. Otra duda sobre la telemetría acústica y su rendimiento a la hora de estimar la actividad está relacionada con la limitada información transmitida por señal acústica. Los acelerómetros acústicos (es decir, los acelerómetros a bordo de los transmisores acústicos) están configurados para registrar la aceleración en tamaños de ventana de tiempo relativamente grandes (normalmente entre 15 y 90 s), y luego calcular un único valor de actividad que es el que se registra en los receptores. Una fórmula muy utilizada para calcular la actividad en ventanas de tiempo es el cuadrado medio

(RMS), cuya idoneidad para estimar la actividad ya ha sido probada. Sin embargo, debido a su naturaleza (raíz cuadrada sobre la suma de las aceleraciones dinámicas al cuadrado para toda la ventana de muestreo, véase la ecuación 1 del Capítulo 1), se plantea la cuestión de su idoneidad en términos generales, ya que el valor de actividad calculado depende del tamaño de la ventana de muestreo. En los próximos años, es de suponer que habrá un aumento exponencial de los estudios de telemetría acústica que utilicen sensores de actividad y, con ello, un aumento de las oportunidades de comparar los datos de actividad entre estudios. En este sentido, sería recomendable diseñar una ecuación más insensible para estimar la actividad, ya que cada estudio probablemente utilizará un tamaño de ventana de muestreo personalizado (es decir, diferente).

Por todo ello, el objetivo de esta tesis es aportar información novedosa sobre el uso de acelerómetros en telemetría acústica que apoye su conveniencia a la hora de evaluar patrones de actividad en animales acuáticos. Este trabajo abarca desde los aspectos más puramente metodológicos hasta el análisis e interpretación de los datos de actividad de los acelerómetros acústicos aplicando los conocimientos adquiridos a lo largo de la tesis. Concretamente, la tesis se estructura en tres capítulos de investigación. En el Capítulo 2 se propone un novedoso marco metodológico para la sincronización de dispositivos que registran datos simultáneamente. En el Capítulo 3 se analiza el rendimiento de los datos de actividad proporcionados por los acelerómetros acústicos a través del uso simultáneo de transmisores acústicos y de un data-logger de aceleración triaxial independiente de alta frecuencia, sincronizando ambos dispositivos gracias al método propuesto en el Capítulo 2. Por último, en el Capítulo 4 se evalúan los efectos ambientales y del buceo sobre los patrones de actividad del mero en un área marina protegida, mejorando la interpretación de los datos obtenidos gracias a los conocimientos adquiridos en el Capítulo 3. Finalmente, en la sección de discusión (Capítulo 5), recogemos toda la información adquirida a lo largo de los diferentes capítulos para evaluar el estado del arte en el estudio de los patrones de actividad de los animales acuáticos mediante telemetría acústica, aportando algunos consejos y destacando las perspectivas de futuro.

En el **Capítulo 2**, utilizamos dos tipos de datos frecuentemente utilizados en biología (aceleraciones y temperaturas), mostrando un ejemplo práctico en el que este método puede ser útil: la sincronización de un acelerómetro a bordo de un transmisor acústico y uno convencional. Además de apoyar la hipótesis en la que se basa el método, los resultados de este trabajo nos permitieron demostrar la fiabilidad del método, ya que fuimos capaces de sincronizar el 100% de los pares de dispositivos analizados. Además, el método demostró su robustez ya que conseguimos sincronizar todos los pares de dispositivos independientemente del tipo de sincronización realizada, observando que el aspecto más crítico para conseguir una correcta sincronización de los pares de dispositivos es el grado de autocorrelación temporal de los datos. Por último, también encontramos una gran variabilidad en los tiempos de sincronización entre los diferentes pares de acelerómetros acústicos y convencionales, mostrando que la desincronización depende de las particularidades de cada par de dispositivos y que es necesario un método que resuelva estos problemas. Hasta donde sabemos, este es el primer trabajo que propone un método de sincronización entre pares de dispositivos fácil de aplicar y que permitiría sincronizar tanto dispositivos desincronizados en el tiempo, como dispositivos desincronizados por problemas técnicos durante el estudio o no sincronizados desde un principio.

En el **Capítulo 3**, combinamos datos de acelerómetros triaxiales de alta frecuencia con datos de telemetría acústica para evaluar y comparar el rendimiento de diferentes variables comúnmente utilizadas como proxies de la actividad en el contexto de la telemetría acústica. Utilizando el mero depredador *Epinephelus marginatus* (Lowe 1834) como especie modelo, evaluamos las posibles consecuencias del uso de acelerómetros acústicos internos, las consecuencias de promediar la actividad en intervalos de tiempo, la existencia de un sesgo de muestreo y el rendimiento de diferentes variables derivadas de la actividad de telemetría acústica: aceleración media, número de detecciones y rango de profundidad, como estimadores de la actividad. Nuestros resultados sugieren que los transmisores acústicos internos proporcionan estimaciones de actividad fiables, especialmente para niveles de actividad bajos. Por último, identificamos algunos posibles problemas al utilizar los datos de aceleración de los transmisores acústicos, como la pérdida de sensibilidad a la alta actividad o el sesgo de muestreo dependiente del individuo, proporcionando consejos y perspectivas para futuros estudios de la actividad de los peces con telemetría acústica.

En el **Capítulo 4**, se evaluó simultáneamente el efecto de las variables ambientales fotoperíodo, hora del día, fase lunar, temperatura, tormentas, intensidad y dirección de las corrientes marinas y presencia de buzos sobre los patrones de actividad del mero (*Epinephelus marginatus*). Para ello, utilizamos datos de aceleración, una zona de control, que nos permitió realizar un diseño estadístico BACI (de las siglas en inglés "Before-After Control-Impact"), y Hidden Markov Models. A la vez que se comprobó la importancia de diversos factores ambientales sobre los patrones de actividad del mero, los resultados también evidenciaron la heterogeneidad de las respuestas en cuanto al tipo y la magnitud de los efectos. Las variables ambientales para las que el mero mostró una respuesta clara y relativamente consistente entre los individuos fueron las tormentas, que aumentaron su actividad, la hora del día, siendo más activos por la noche que por el día, la temperatura o la intensidad de la corriente, cuyos aumentos desencadenaron incrementos en la actividad, y la fase lunar, con tendencias a reducir la actividad a medida que se acerca la luna llena. Sorprendentemente, algunos de los resultados obtenidos difieren de los supuestos ampliamente aceptados para esta especie en cuanto a sus patrones de actividad, lo que pone de manifiesto la importancia de los avances en biología y la necesidad de nuevos estudios incluso en líneas de investigación ya abordadas, pero con metodologías inadecuadas. Creemos firmemente que este estudio aporta el conocimiento más fiable hasta la fecha sobre los patrones de actividad del mero, una especie de gran interés pesquero, además de ecológico y recreativo, que puede ayudar a su gestión sostenible combinando la conservación de los ecosistemas con el disfrute y apreciación de la naturaleza, y la viabilidad de la actividad pesquera artesanal.

Los resultados de la tesis doctoral nos permiten ampliar el conocimiento sobre el funcionamiento y, por tanto, interpretación de los datos obtenidos por medio de la telemetría acústica a la hora de inferir actividad. En el caso del mero, por ejemplo, el conocimiento ya adquirido podría empezar a aplicarse para la gestión de las poblaciones de mero, ya que puede ayudar a diseñar medidas de gestión eficaces que combinen su conservación con su disfrute por parte del turismo de naturaleza y su explotación por parte del sector pesquero (profesional o recreativo). No obstante, tal y como se señala a lo largo de la tesis, la misma sirve también como punto de partida para futuras líneas de investigación que ayuden a mejorar los conocimientos actuales sobre la temática abordada, todavía bastante limitados.

Chapter I

General Introduction

1. Animal behaviour in the aquatic environment

The study of animal behaviour plays a key role in multiple disciplines for a variety of reasons. For example, it has been shown that predator behaviour shapes certain ecosystems since it changes the behaviour of species situated below in the trophic chain (e.g., Fortin *et al.* 2005, Catano *et al.* 2016, Nagelkerken & Munday 2016). The understanding of animal behaviour has also proved to be useful in the context of disease transmission, since this knowledge can help designing measures that reduce the spread of infectious agents (Smith & Harris 1991, Craft 2015, de Freslon *et al.* 2019). Likewise, knowledge about species spatial or breeding behaviour and how they respond against different environmental factors is crucial for conservation purposes (Tucker *et al.* 2018). As the last example, understanding animal behaviour can also be useful in economic terms, as it helps to either improve animal welfare or to adjust farm practices (e.g., Terrasson *et al.* 2016, Føre *et al.* 2017, Muñoz *et al.* 2020).

The study of animal behaviour, however, is complex for several reasons. One aspect that hampers the study of animal behaviour is related to technology involved in data-acquisition. Nonetheless, the constraints are different among technologies and are usually greater in aquatic than in terrestrial environments due to the physico-chemical conditions imposed by the water. Data collection methods based on biologgers, for example, implies the need for re-catching individuals after a certain time from the implantation of recording devices (Cooke *et al.* 2012). This implies higher sampling efforts and the constraint to use them with species of easy re-capture. It might be even necessary to increase the sample size (number of tagged individuals) to account for the likely loss of devices. In contrast, telemetry methods avoid the need for re-capture individuals, since data are directly transmitted to receivers (e.g., hydrophones or satellites). Devices that transmit data are called transmitters, and they usually do it wirelessly, mostly using electromagnetic or acoustic signals (Cooke *et al.* 2016). However, telemetry technologies have other limitations. Firstly, they are more expensive than methods such as biologgers, visual census or video-recording. Secondly, the data acquisition might depend on the animal position regarding the receivers, leading to data-gaps when, for instance, animals are hidden and the signal does not reach the receivers. A third constraint is associated with battery lifespan. Part of the energy from transmitters batteries is used to send the signals, which reduces the tracked time for individuals compared to other non-telemetry methods like biologgers. Another important constrain of telemetry technologies is related to data-transmission ability. Some types of transmitters can send a limited amount of information on each signal, impacting both the quality and the quantity of data obtained. An example is the use of acoustic transmitters equipped with both a temperature and a depth sensor: these devices cannot send temperature and depth data simultaneously but must be sent separately, which reduces the number of data obtained from each type. Finally, another constraint of telemetry methods is the size of the transmitters. Some of them (e.g., satellite transmitters) are relatively large, which prevents their use in small species. In short, it is crucial to choose a data collection method according to the particularities of our study (e.g., target-species, environmental conditions, goals, etc.), as this will determine the type of the data obtained (Cooke *et al.* 2012, Brownscombe *et al.* 2019). For example, it is not advisable to use biologgers in hard-to-catch species because of their behaviour or the environmental conditions. On the contrary, acoustic telemetry might not be adequate in highly mobile aquatic species (e.g., migratory) if the area where receivers area arranged is small compared to their movement area, or satellite telemetry may be inappropriate in small aquatic species or those who do not reach the water surface.

Another aspect that complicates the study of animal behaviour is associated with the difference in behaviour among individuals, as it is well known that data collection by certain sampling methodologies (e.g., camera-traps or acoustic telemetry) might be affected by animal behaviour (e.g., Stuber *et al.* 2013). For instance, Glen *et al.* (2014) showed that different species could have different detection probabilities associated with their movement speed. In line with that, it is likely that individuals of the same species with different individual behaviour might also have different detection probabilities when using technologies such as camera-traps or acoustic telemetry, generating a sampling bias (Caravaggi *et al.* 2020). The increasing evidence about the importance of intraspecific behavioural differences supports this idea (e.g., Dingemanse *et al.* 2010, Montiglio *et al.* 2013). On the other hand, intraspecific behaviour variance

might also imply greater difficulties in finding general patterns, especially if few individuals are monitored, hampering the design of conservation measures.
Activity is one of the most widely approached issues in animal behaviour studies (e.g., Réale *et al.* 2007, Wolf & Weissing 2012, Sih *et al.* 2015). Immelmann & Beer (1989) defined activity, in an ethological sense, as "an individual animal's general, or specific, movement". The importance of this topic and the broad nature of this term have led to thousands of studies in which the activity of animals has been assessed in one or another way. One of the most novel methods of inferring activity in wild animals is through accelerometers (Halsey *et al.* 2011, Wilson *et al.* 2019). Accelerometers are devices that measure a body acceleration, usually in its three space dimensions (x, y, z), although some measure it in two. A tri-axial accelerometer perfectly calibrated and static upon any surface would measure 9.8 m s^{-2} between the three axes. When these devices are fixed to the animals' body, the three axes acceleration values help understand what kind of movements (i.e., behaviours) they perform. Also, it proved to be valid for inferring activity in numerous species given the close relationship between acceleration and energy cost (Wilson *et al.* 2019). One issue that has become particularly relevant in recent years is the impact of human-induced change in animal activity, both spatially and temporally (Wilson *et al.* 2020). For example, Benítez-López (2018) and Gaynor *et al.* (2018) show how human activities are driving temporal drifts in the activity patterns of numerous mammal species, which tend to increase their nocturnality as a response to human disturbance. As Wilson *et al.* (2020) stated, human activities affect the species differently for myriad reasons (differences in animal personality, the temporal distribution of human activities, etc.), and might cause impacts at the community level by altering the species co-occurrence and their interactions. A group of species in which the human activity especially impacts activity patterns is that of predators, which arise a major interest for humans and are commonly more vulnerable to shifts in community composition (Woodroffe 2000, Kruuk 2002, Sergio *et al.* 2008).

Acoustic telemetry has become one of the leading technologies for studying animal behaviour in the aquatic realm, including activity patterns (Hussey *et al.* 2015). This is due to the good propagation properties of acoustic waves in the aquatic environment, enabling the easy development of a network of transmitters and receivers. In contrast, the propagation ability of electromagnetic waves in the aquatic environment is weak, restricting the use of satellite transmitters to species living in close contact with the water surface (Cooke *et al.* 2012). This can be solved with pop-up satellite transmitters, although they are larger and more expensive. However, both acoustic and satellite telemetry can be used in the aquatic environment, although each of them is advisable for different situations (Cooke *et al.* 2012). Broadly speaking, acoustic telemetry is advisable for aquatic species far from the water surface (e.g., benthic species), with known movement areas that can be covered by the set of receivers at our disposal. In contrast, satellite telemetry is usually more useful for highly mobile species (e.g., migratory) that are closely related to the water surface, allowing electromagnetic signals to reach satellites. A clear example of the usefulness of acoustic telemetry in studying aquatic animal behaviour is provided by the benthic fish species dusky grouper (*Epinephelus marginatus*, Lowe 1834). Thanks to this technology, it has been possible to confirm the pronounced sedentary behaviour of the dusky grouper, a species that usually moves within relatively small areas (e.g., Lembo *et al.* 1999, 2002, Spedicato *et al.* 2003). This information has, for example, helped to strengthen the arguments for the creation of marine protected areas (MPAs) even when they are small, as they could protect the habitats of this extremely ecologically and socio-economically valuable species (Giakoumki *et al.* 2017, Di Franco *et al.* 2018). However, there are still many aspects of the behaviour of this emblematic species to be explored, and that could be crucial for its conservation (Condini *et al.* 2018). In line with stated earlier, one topic little known but of special relevance is the human-induced behavioural changes in dusky groupers, especially those produced by the scuba-diving activity. Recreational diving is an activity that has grown exponentially in the last decade due to society's increasing interest in nature-based tourism (Geffroy *et al.* 2015). Besides, this activity tends to be concentrated in protected areas due to their conservation degree, associated with higher species-richness and landscape value (Willemen *et al.* 2015). In the marine environment, these sites correspond to the MPAs, especially effective areas for the conservation of species such as groupers (Harmelin-Vivien *et al.* 2007, Anderson *et al.* 2014, Hackradt *et al.* 2014) and where currently scuba-diving activity is quite intense (Cerrano *et al.* 2017). However, despite the ecological importance of groupers in the ecosystems they inhabit, it is still unknown how this activity can affect their behaviour. In this sense, acoustic telemetry is a handy tool that can help to understand such effects (Brownscombe *et al.* 2019). However, at present, there is no work to our knowledge that evaluates the effect of scuba diving on the activity patterns of aquatic animals using acoustic telemetry. There is only one study performed on the species *Carcharhinus amblyrhynchos* (grey reef shark) (Bradley *et al.* 2017), but it does not assess changes in activity patterns but in spatial ones.

2. Acoustic telemetry performance when assessing aquatic animals activity: constraints and uncertainties

Despite the usefulness of acoustic telemetry in assessing aquatic animal activity, it has a number of technical limitations that are important to consider both when designing studies and when interpreting the data. On the one hand, when indirect measures of activity are used, such as the number of detections per time bin (Skerritt *et al.* 2015), it must be considered that this value can be highly affected by environmental conditions (e.g., temperature, current intensity, environmental noise, etc.) (Payne *et al.* 2010, Gjelland & Hedger 2013, Huveneers *et al.* 2016). Hence, the variation in the number of detections could result from the variation in environmental conditions and not of the animal activity. Another example of an indirect measure of activity would be the distances travelled (Villegas-Ríos *et al.* 2014). In this case, an important limitation could be the spatial resolution, which is very variable between studies depending on the design-type (Brownscombe *et al.* 2019). Thus, a species can be very active within small areas (e.g., tens of metres) that are below the spatial resolution level of our study (e.g., hundreds of metres or kilometres), and conversely, make greater movements in periods of less overall activity. On the other hand, even when using direct measurements of activity through accelerometers coupled to transmitters, there are a series of technical limitations to be considered when evaluating activity (Murchie *et al.* 2011): (a) the detection of the acoustic signal depends on the animal position relative to the receivers, (b) data-recording is intermittent, (c) the accelerometers usually have narrow acceleration ranges compared to conventional accelerometers as well as a low recording frequency, and (d) activity is estimated for large time-windows (e.g., 30 s), losing temporal resolution.

Associated with these technical limitations, there are several doubts about the performance of acoustic telemetry that has not yet been fully clarified. Firstly, acoustic transmitters are usually placed inside animal body (e.g., coelomic cavity) to avoid biofouling and their possible loss through detachment when they operate for long time periods (~ months) (Cooke *et al.* 2012, Brownscombe *et al.* 2019). This implies that transmitters are left with free-ranging movement since they cannot be fixed inside the animal body, raising the uncertainty about the effect this may have when comparing data among individuals (Wilson *et al.* 2019). Another concern that arises because of acoustic telemetry constraints is the existence of sampling-bias issues. Variation in animal behaviour is usually explained, at least in part, by the type of habitat in which they are found (e.g., Godvik *et al.* 2009, Wolf & Weissing 2012). In this sense, it is likely that the intensity of animal activity may also change among habitats. This can be a problem when using acoustic telemetry, as their performance is highly dependent on abiotic factors such as the habitat type (associated with the presence of barriers to sound propagation) (Huveneers *et al.* 2016). Therefore, the probability of detecting acoustic signals from transmitters may vary depending on the habitat in which the individual is present, which results in a sampling bias. If, for example, individuals were more detectable in habitats where they are more active, we would obtain a higher proportion of high activity values concerning the real one. Another doubt arises from the intermittent nature of data. In occasions, it is necessary or advisable to average raw activity values over large time bins (e.g., 15 min, 30 min, etc.) (Taylor *et al.* 2013, Udyawer *et al.* 2017, Runde *et al.* 2020). However, at increasingly time bin sizes there is a greater risk of sampling activity just for a small proportion of time concerning the total time, which poses the uncertainty about the consequences of using different time bin sizes. Another doubt about acoustic telemetry and its performance when estimating activity is related to the limited information transmitted per acoustic signal. The acoustic accelerometers (i.e., accelerometers onboard acoustic transmitters) are configured to record acceleration in relatively large time-window sizes (normally between 15 and 90 s), and then calculating a single activity value which is what is recorded on the receivers. A widely used formula for calculating activity into time windows is the root mean square (RMS), whose suitability to estimate activity has been already tested (Wilson *et al.* 2013). However, due to its nature (square root over the sum of dynamic accelerations squared for the whole sampling window, see Eq.1 in Chapter 1), the question arises about its suitability in general terms, since the calculated activity value is sampling-window-size dependent. In the coming years, there will presumably be an exponential increase in acoustic telemetry studies using activity sensors and with that, a rise in the opportunities to compare activity data between studies (Cooke *et al.* 2016). In this sense, it would be advisable to design a more insensitive equation to estimate activity, since each study will likely use a customized (i.e., different) sampling-window size.

In summary, although the advances in acoustic telemetry in recent years have offered a novel methodological framework for studying aquatic animal activity, information about the possible effects of its limitations is scarce. This knowledge is crucial since it would improve the interpretation of these data. One possible reason for such an information shortage is the difficulty of synchronizing the acoustic transmitters with other independent accelerometers in natural environments, a methodological approach that would undoubtedly be very useful. The time associated with each detection downloaded by acoustic receivers does not correspond with either the start or end recording-time, but with the time in which the acoustic signal reaches the receiver. Thus, given that the time lapse between the acoustic signal reception and the recording ending-time might vary depending on several factors (e.g., the animal's distance from the receiver, acoustic reverberations, water's properties, etc.), it becomes challenging to calculate the exact sampling-window in which acoustic accelerometers recorded acceleration. Also, accelerometers (acoustic and non-acoustic ones), like any other device with a clock, suffer from time drifts that can be of different magnitude and direction even among devices from the same model (Packard 1997, Zhou *et al.* 2008, Tirado-Andrés & Araujo 2019), being of special relevance in marine environments where they might suffer sharp changes in temperature and pressure conditions associated to the animals' vertical movement. As previously mentioned, all this together is likely to have complicated the development of methodological works in which activity data from acoustic accelerometers are assessed by comparing them with data from ordinary (i.e., non-acoustic) accelerometers.

3. Aim of the Thesis

The study of activity patterns is a key point in ethology. Until recently, acoustic telemetry, one of the leading technologies in this field, only allowed their evaluation using indirect measurements (e.g., number of detections or distances travelled per time interval). In this sense, the addition in the last decade of accelerometers to the range of sensors available in acoustic transmitters has represented a great advance, given the direct relationship between acceleration and energy expenditure (Wilson *et al.* 2019). However, activity data obtained from acoustic accelerometers have very particular characteristics due to the inherent limitations of acoustic telemetry technology when applied in natural environments. Despite this, there is a lack of studies that evaluate the suitability of data obtained by acoustic accelerometers in natural environments.

This thesis aims to provide novel information about the use of accelerometers in acoustic telemetry that supports their convenience when assessing activity patterns in aquatic animals. This work ranges from more purely methodological aspects to analysing and interpreting activity data from acoustic accelerometers applying the knowledge gained throughout the thesis. Concretely, the thesis is structured in 3 research chapters. Chapter 2 proposes a novel methodological framework for synchronising devices that record data simultaneously. Chapter 3 analyses the performance of the activity data provided by acoustic accelerometers throughout the simultaneous use of acoustic transmitters and an independent high frequency tri-axial acceleration data-logger, synchronising both devices thanks to the method proposed in Chapter 1. Lastly, in Chapter 4, we evaluate environmental and scuba-diving effects on the activity patterns of dusky grouper in a marine protected area, improving our interpretation of the data obtained thanks to the gained knowledge in Chapter 3. Finally, in the discussion section (Chapter 5), we gather all the information acquired throughout the different chapters to assess the state of the art in studying activity patterns of aquatic animals using acoustic telemetry, providing some advice and highlighting future perspectives.

Chapter II

A simple method for synchronising data-loggers used simultaneously under changing and unpredictable environmental conditions

A simple method for synchronising data-loggers used simultaneously under changing and unpredictable environmental conditions

ABSTRACT

Technological advances in the last decade concerning electronic tags (data-loggers and transmitters) and the drop in their prices have triggered a vertiginous boom in studies using this data source. A scientific field in which this has been especially evident is in the animal behaviour one, in which data obtained through animals borne devices have led to important findings. On occasions, some of these studies require the simultaneous use of electronic tags over time. However, the analysis of data recorded simultaneously by different devices requires synchronising these devices, something very complex at times due to the unpredictability of the drift of their internal clocks. This becomes an especially severe problem when studying aquatic species that move vertically in the water column, thus exposing the devices to a wide range of environmental conditions. Therefore, in this study, we propose a method of synchronizing devices that record data simultaneously over time, based on the hypothesis that if the data over time from the sensors are correlated to some degree, their maximum correlation will be when they are synchronised. For this purpose, we tested the method in several case studies (pairs of accelerometers located in different parts of a human’s body, pairs of temperature sensors placed along a depth gradient, and pairs of accelerometers of different nature [acceleration biologger and acceleration transmitter] attached to a fish species). The method succeeds in synchronising all the pairs of devices independently of the type of synchronization performed. Great variability in the synchronization times was observed among the different pairs of acceleration transmitter and acceleration biologger, showing that the desynchronization depends on the particularities of each pair of devices. The degree of temporal autocorrelation of the data is the most critical aspect of the correct synchronization of the pairs of devices. In conclusion, here, we demonstrate the reliability and flexibility of the proposed synchronisation method, making it potentially useful in future studies that use different electronic tags simultaneously.

1. Introduction

The use of simultaneous sensor data (e.g., temperature, pressure, sound, light, etc.) over time is common in many scientific disciplines, such as computer science, medicine or animal behaviour, and for a variety of reasons (e.g., sensor calibration, clinical monitoring, animal welfare, etc.) (Müller & Schrader 2003, Douaik *et al*. 2006, Henocq *et al*. 2010, Yang *et al*. 2015, Hughey *et al*. 2018). The benefits of using simultaneous data become especially useful in ethological studies, to better understand how certain animal behaviours (position, depth, activity, movement, sound, etc.) are affected by different extrinsic factors (light, temperature, oxygen concentration, humidity, etc.) (Benton 2009, Shamoun-Baranes *et al*. 2012, McClintock *et al*. 2013, Hughey *et al*. 2018). This, coupled with the rapid technological advances and the drop in the price of electronic tags of recent years, has triggered a significant increase in the volume of studies using simultaneous data from animals (e.g., Furukawa *et al*. 2011, Evans *et al*. 2013, Photopoulou *et al*. 2015, Miller & Dowd 2017, Coffey *et al*. 2020). The collection of data on animals, however, is sometimes complex, since in many cases, the data obtained varies depending on the part of the body on which the device is located and the type of data-logger used (Sellier *et al*. 2014). Therefore, it is often useful to collect simultaneous data to compare how the data from a sensor varies depending on its placement on the body or what are the consequences of using different recording-settings (Müller & Schrader 2003, Viviant *et al*. 2010, Nishiumi *et al*. 2018, Coviello *et al*. 2020).

A problem that typically arises when obtaining simultaneous data from different devices, is the need for time synchronisation between them. The most studied cause of desynchronisation within a sensor network is associated with the clocks drift. Any clock has what is called an inherent "drift" from the international atomic time (TAI from the French name “temps atomique international”). This means that over time, the time estimate of any given clock moves away from that “reference time”, either forward or backwards. The magnitude of these drifts changes over time due to extrinsic factors such as temperature or pressure conditions, but also for intrinsic factors like clock ageing or the type of material the clock is made of (Packard 1997, Zhou *et al*. 2008, Tirado-Andrés & Araujo 2019). Although less studied, another cause of time desynchronisation in sensor networks is related to the internal functioning of the sensors (Klein *et al*. 2011, Gaylord & Sanchez 2014). Thus, even clocks from the same model can perfectly drift differently, especially if they are under different environmental conditions. The research efforts of the last decades to ascertain the causes and consequences of clocks drift have enabled to pose different solutions to this problem in multiple scenarios (Marouani & Dagenais 2008, Klein *et al*. 2011, Hannemann *et al*. 2013, Jiang *et al*. 2018, Steel *et al*. 2019, Coviello *et al*. 2020).

Although the requirements on time synchronisation accuracy when using simultaneous data may vary depending on the goals of the study and the data-type (its frequency [s^{-1}, min^{-1}, h^{-1}], etc.), it is always necessary to ensure that both devices are synchronised to some degree to warrant that we compare data taken at the same reference time. Unfortunately, the accurate synchronisation of devices might become a problem of special concern for different reasons. First, with numerous devices, it is impossible to guarantee devices synchronisation at a tenth of a second scale because of their time configuration type (e.g., manually disconnecting the device from a computer). Second, specific environments like aquatic ones pose additional constraints when synchronising devices, since temperature and pressure conditions are constantly changing, especially if they are attached to animals. In addition, one of the most commonly used technologies in those environments, the acoustic telemetry (Hussey *et al*. 2015), has two additional limitations when synchronising devices; on the one hand, the date and time obtained for the recording of the acoustic transmitter are those of the detection at the acoustic receiver, which makes it impossible to calculate accurately the time for which the acoustic transmitter sensor was recording. On the other hand, the acoustic receiver and any other device are usually synchronised days before operating in the field, moment from which they start to desynchronise. In that sense, the specificity of this issue, together with the complexity of the studies dealing with the correction of time drift, have probably led to the absence of alternatives when it comes to synchronizing devices used simultaneously in animals. Therefore, we aim to propose a novel synchronisation method between pairs electronic tags, either data-loggers or transmitters. It is especially useful in aquatic animals, which can move considerably in the water column with the resulting consequences in terms of clocks-drift associated with changes in pressure and temperature, among other environmental parameters. The application of this method assumes that if the data over time from the sensors are correlated to some degree, the maximum degree of correlation will be when they are synchronised. Here we used acceleration and temperature data because of their relative importance in ecological and ethological studies (e.g. Müller & Schrader 2003, Viviant *et al*. 2010, Yasuda *et al*. 2010, Moland *et al*. 2011, Gutowsky *et al*. 2017). The advantages of this method are (i) its ease of application, since it is not necessary to model the drift of the internal clock of the sensors, nor to know their internal functioning (as it uses data from both devices to derive the time drift between the devices); and (ii) that it allows synchronising sensors that for some reason were not perfectly synchronised from the beginning or that could have become desynchronised during the study period. Concerning the latter, it should be noted that the drift of the internal clock of a sensor starts from the moment the time is set, even when the device is switched off afterwards, as the clock continues to operate. Therefore, if two devices are synchronised, but the experiment is performed later (days, weeks, etc.), they will most likely be desynchronised. Thus, the proposed method can be useful in a wide range of scenarios.

Our specific goals were to (1) propose and test a simple method to synchronize data-loggers used to collect simultaneous data, (2) assess which aspects of the data or which configurable parameters of the method are important for the feasibility and accuracy of the synchronisation, and (3) show some scenarios in which this method might be helpful.

2. Materials & Methods

2.1 Equipment

We used three different data sources: a tri-axial acceleration biologger (model X16-mini, Gulf Coast Data Concepts Inc.), a tri-axial accelerometer onboard an acoustic transmitter (model V13AP, Vemco Inc.) and a water temperature data-logger (model HOBO Water Temp Pro v2, Onset Inc). Accelerometers measure proper acceleration, usually in the three space-axis (X, Y, Z), recording 9.8 m s^{-2} among the three-axis when they are static. The X16-mini records acceleration in continuous, it can be configured to record acceleration at different frequencies (1 to 800 Hz) and has an acceleration sensitivity range of ± 16G (1G ≈ 9.8 m s^{-2}). Data are downloaded directly from the device by connecting it to a computer via a USB cable and the time is configured manually (i.e., the desired initial time starts when the device is disconnected from the computer). As for the V13AP, it is an acoustic transmitter (i.e., recorded data is sent by an acoustic signal to a receiver from where it is downloaded the data) that incorporates an accelerometer (henceforth “acceleration transmitter”) and a depth sensor. Acoustic transmitters are commonly used in aquatic environments, where the use of alternative methods like archival or Satellite tags is challenging given their constraints (Cooke *et al*. 2012, Donaldson *et al*. 2014, Hussey *et al*. 2015). Each acoustic signal consists of an ID and a sensor value in the case the acoustic tag is equipped with one, and the date and time of its reception at the receiver (not of its recording at the sensor) are stored. The acceleration range of the acceleration transmitter onboard the V13AP is ± 3G. Similarly to acceleration transmitters from other companies, these do not record acceleration continuously but are set to record acceleration intermittently. The time-windows for which the acceleration transmitters record acceleration are usually referred to as "sampling-windows" and the time-lapse among consecutive data recording "sampling-delay". For Vemco acceleration transmitters, the sampling-window size (SWS) is constant over time, while the sampling-delay is random, which means that the time-lapse changes among consecutive acceleration records. Both the SWS as the sampling-delay are typically decided by the researchers. In the sampling-delay case, what is usually defined is the average sampling-delay (ASD), i.e. the mean delay among consecutive data recording, and the sampling-delay range (SDR), which represents the fixed time range within which the sampling-delay can oscillate. Once the acoustic accelerometers have recorded acceleration for a given SWS, they calculate a single activity value transmitted. Hence, researchers cannot collect raw accelerations when using acoustic telemetry but an activity estimate for a relatively wide time interval given by the sampling-window size. Among the different equations available for calculating this activity value, the accelerometer from the V13AP uses the root mean square (RMS) formula (Eq. 1) (Cooke *et al*. 2016):

$$RMS = \frac{\sqrt{\sum_{i=1}^{n}\left(X_{dyn}^2+Y_{dyn}^2+Z_{dyn}^2\right)}}{n}\ (Eq. 1),$$

where X_{dyn}, Y_{dyn} and Z_{dyn} represent the dynamic acceleration (m s^{-2}) in the X, Y and Z axes, respectively and *n* the number of acceleration measurements (given by the SWS and the recording frequency [in Hz]). Although the acceleration transmitters record activity irregularly, we can calculate the exact time window for which they recorded acceleration if we have the previous depth record, as they start recording acceleration immediately after the depth info transmission. The HOBO sensor measures temperature in a range between -40º C and 50º C, and it can be configured to record it at frequencies between one second and eighteen hours. In this study, the acceleration biologgers were set to record acceleration at a frequency of 12 Hz, while the acceleration transmitters recorded it at 5 Hz, with an SDR between 120 and 360 s and an ASD of 240 s, and an SWS of 57 s; and the HOBO sensor recorded water temperature hourly.

2.2 Data acquisition

2.2.1 Case study 1: Acceleration data from data-loggers placed simultaneously on humans

We obtained simultaneous acceleration data from two acceleration biologgers (model X16-mini) in two different scenarios, in order to evaluate the synchronisation effectiveness when acceleration measures of two accelerometers are more or less correlated. In the first scenario (“María”), we placed one accelerometer in the upper arm and the other in the forearm of a person (Fig. 2.1A). In the second, less correlated scenario (“Antonio”), we placed one accelerometer in the upper arm and the other in the shin of another person. The programmed time delay between devices was approximately 30 s and 90 s for María’s and Antonio’s cases, respectively. We could not determine the exact time delay between data-loggers at a resolution of tenths of a second since the time on these devices is set manually when disconnected from a computer via a USB cable. In both cases, the data-loggers were configured to record acceleration at 12 Hz.

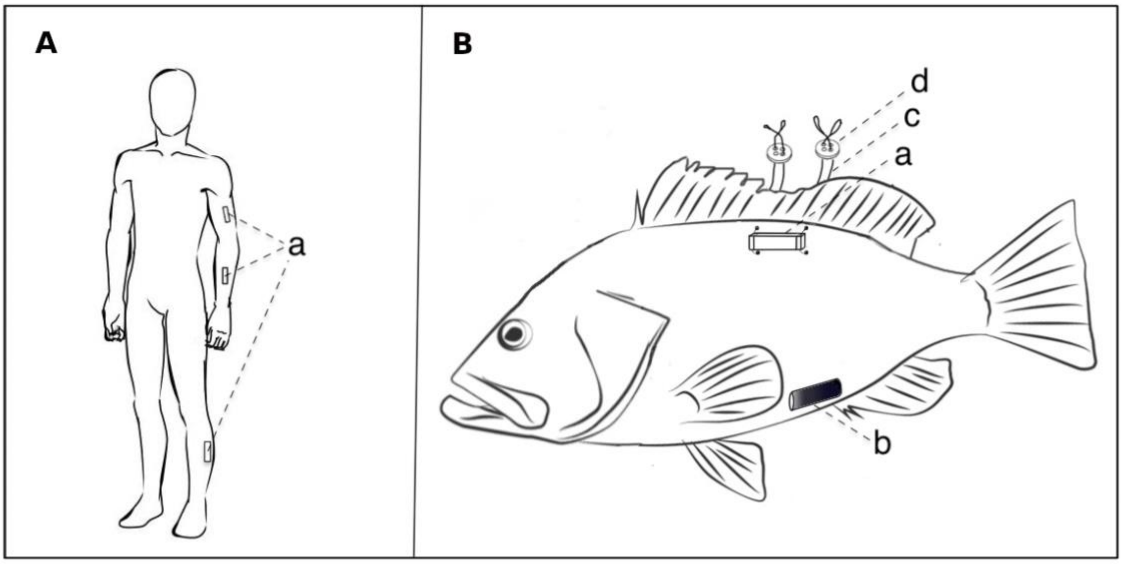

Figure 2.1. Schematic representation of the position in which the devices were placed on both the humans (A) and the dusky groupers (B). a: acceleration biologger (model X16-mini, GCDC Inc.); b: acceleration transmitter (model V13AP, VEMCO Inc.); c: biodegradable monofilament surgical suture; d: sterile button.

2.2.2 Case study 2: Temperature data from four data-loggers placed simultaneously at different depths in a marine protected area

Hourly water temperature data were gathered from four synchronised temperature sensors placed simultaneously at different depths (5, 15, 25 and 35 meters) in a specific site of the marine reserve of Cabo de Palos - Islas Hormigas (37° 39.316’ N, 000° 38.952’ W). The sensors were fixed to a rope that was anchored at its lower end to a mooring of concrete and at its upper end carried a buoy that allowed the rope to be vertical in the water column.

2.2.3 Case study 3: Real study case with acceleration data from an acoustic transmitter and a conventional accelerometer

Finally, we collected simultaneous acceleration data from two different electronic tags placed in six wild individuals of the fish species dusky grouper (*Epinephelus marginatus*, Lowe 1834). One of the tags was a tri-axial acceleration biologger (model X16-mini) and the other an acoustic transmitter (model V13AP) which incorporates a tri-axial accelerometer. The acceleration biologger was placed externally close to the dorsal fin and the acceleration transmitter internally in the ventral fish cavity (Fig. 2.1B). The acceleration biologger was set to record acceleration continuously at 12 Hz. For its part, the acceleration transmitter recorded acceleration intermittently using a fixed SWS of 57 s and with an average time delay of 240 s but within a restricted time-range of ±120 s around it (i.e., the minimum time between consecutive activity values is 120 s while the maximum is 360 s). Individuals were caught using artisanal baited traps, a technique that avoids the damage and stress generated using line and hook. Once fish were on board, they were placed in a tank with seawater regularly aerated and replaced. The swim bladder was deflated with a hollow needle prior to handling. Once stabilized, fish were moved to other tank containing the anaesthetic solution (40 mg/L; 1-part clove oil emulsified in 9 parts ethanol). The anaesthesia procedure followed ethical recommendations by Neiffer & Stamper (2009). Fish were maintained in the anaesthetic solution until loss of balance and lack of response when pressing the caudal peduncle was observed. Once anaesthetized, they were placed on an ichthyometer for the surgery. The head was covered with a wet towel and cold seawater was run through the gills during the surgery. First, we made a 3-cm incision in the belly using a sterile surgical blade to access the ventral cavity, where we introduced the V13AP tag. After that, we sealed the incision using surgical glue to avoid the permeability of the wound. As for the acceleration biologgers, they were fixed externally close to the dorsal fin (Fig. 2.1B). The acceleration biologgers were encapsulated with heat-shrink tubing following Ciancio *et al*. (2016), and attached to the animal with biodegradable monofilament surgical suture (Monomax 1 HR37, B. Braun Inc) and two sterile buttons. This type of attachment was performed to be sure that the devices would be released if we were not able to recapture the individuals, although it allowed some animals to hold them for up to two months after placement (Pereñíguez, J.M., pers. obs.). The total weight (in the air) of our devices was approximately 16 g and 13 g for the acceleration biologger and the acceleration transmitter respectively, and therefore, they never exceeded 1% of the body mass of individuals. Povidone-iodine and an antibiotic spray were spread on the manipulated area. The transmitters were always placed keeping the same orientation in all individuals. After a short recovery, fish were released in the same area where they had been captured. Fish behaviour was checked by a diver for at least 10 minutes from their released at the surface to the moment in which they leaned on the bottom or they hid in some cave. The external accelerometers were recovered through fish recapture (using baited traps) between 14 and 45 days after releasing, liberating individuals in the same area where they were captured, with no abnormal behaviour signs. Handling and tagging procedures followed recommendations described by Wagner *et al*. (2011) and were performed by trained and authorized personnel. We also had the pertinent permits, both for working in protected areas and for ethical aspects related to animal welfare.

2.3 Synchronisation method

The synchronisation method consists of measuring the correlation strength between two devices data over time, changing in one of them (settable device) the time-setting while always keeping fixed the time-setting for the other (reference device). In this study, ‘time delay’ refers to the time between the original time for the settable device and the selected time when measuring the correlation strength between devices data; for its part, ‘time-delay-range’ (TDR) refers to the time range within which the settable device time-setting is changed, while time-delay-resolution’ (TDRes) is the time-lapse among distinct time delays used. For instance, a TDR of ± 60 s with a TDRes of 1 s implies that we used 121 different time-settings for the settable device. Once we have measures of some physico-chemical quantities (e.g., acceleration or temperature) for two devices for the same period, the general procedure is always as follows, independently of the type of data (see Fig. 2.2 for further details): (i) we delimit the period for which we can sample data in both data-loggers to ensure that it is never sampled in periods of no data for the settable device (Fig. 2.2B), (ii) we select specific data-windows over time from both devices using a fixed SWS and a customised random sampling-delay defined with its SDR, (iii) we used either the raw data from the selected sampling-windows or an statistic (e.g. RMS, mean, etc.) calculated for each sampling-window, (iv) we calculate the correlation strength between data obtained by the two devices, and (v) we repeat the process from step ii to step iv changing the time for the settable device in customised TDR and TDRes values to see for which time delay in the settable device the highest correlation between devices data occurs.

In Appendix A and Appendix B, we share an R-code to perform the proposed synchronisation procedure using the R software environment. This code follows the procedure described above and allows easily configuring the parameters SWS, SDR, Treatment, TDR, and TDRes, enabling to accomplish the synchronisation in different ways depending on each case-particularity (correlation strength between devices data, time period, temporal resolution, etc.). It also allows using three different statistical methods for measuring the correlation strength between devices data (Pearson's, Spearman's and linear model). Once the code is run, it automatically creates a ".csv" file in the indicated directory where the correlation strength between devices data for different time delays for the settable device appears. Thus, the next step would be to change the time-setting manually for the settable device according to the synchronisation time calculated with the R-code, which follows, as we mentioned previously, the procedure described above. The code also allows using complete temporal data series (i.e., do not select specific sampling-windows) through an SDR of 0, which might not be advisable in certain circumstances such as overwhelming data volumes that may excessively slow down the R software functioning or when data have high temporal autocorrelation (e.g., >0.9). Lastly, the code also enables to treat or not the data from the selected sampling-windows, which might be advantageous in certain circumstances, such as case study 3 (activity data from acoustic transmitters), where sensors’ data have been treated before their transmission.

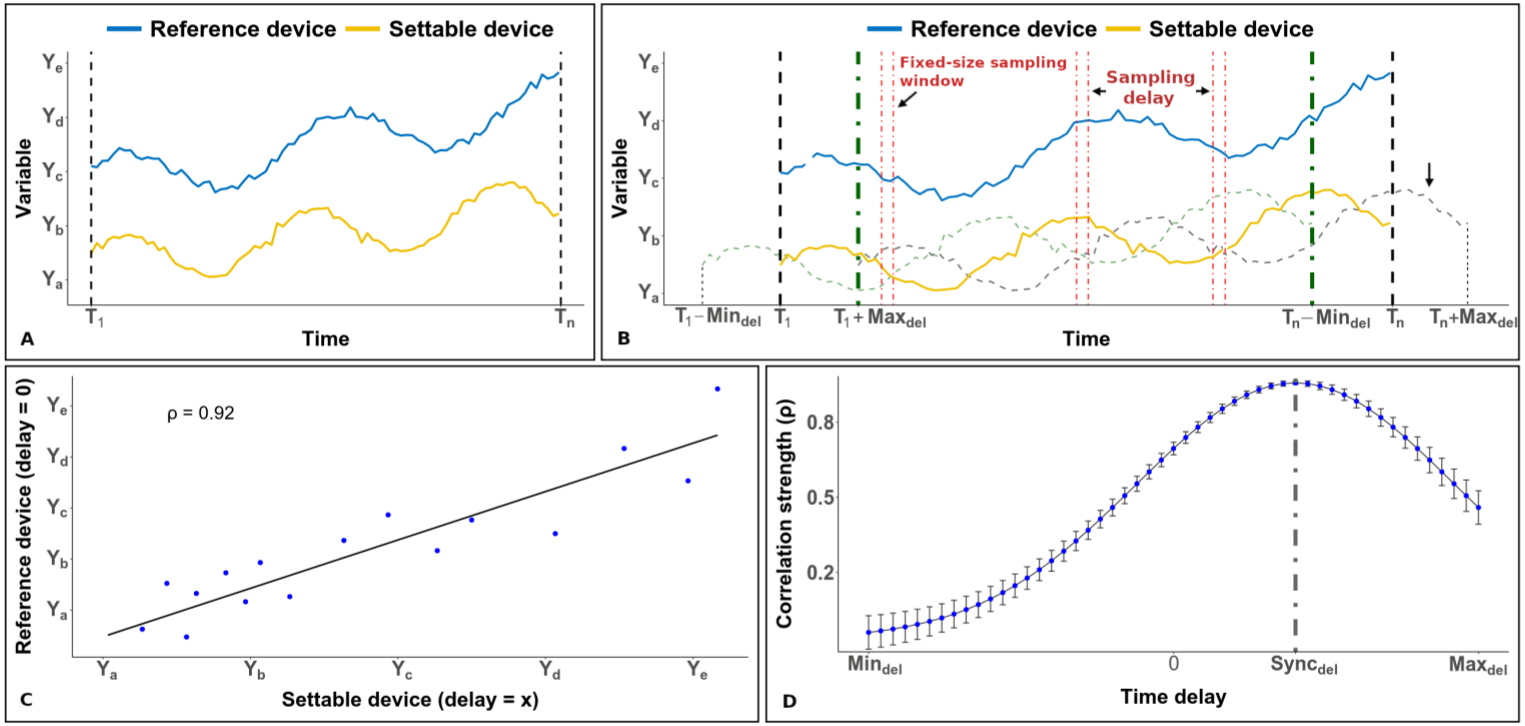

Figure 2.2. Schematic representation of the synchronisation process proposed: We start by having correlated simultaneous sensors' data (A). Then, we select data at fixed-size sampling-windows (vertical red dashed lines), using a random sampling-delay, and within a specific time interval (vertical green dashed lines) for which we know there is data for both sensors and which is determined at its extremes by the maximum time added (Max_{del}) or the minimum time subtracted (Min_{del}) to the initial (T_1) or final (T_n) original-time of the settable device, respectively. Afterwards, we measure the correlation strength between data selected (i.e., data from the sampling windows) from both devices (C) and repeat the sampling process n times to obtain a mean correlation value with its associated error measurement (D). We repeat this process changing the time for the settable device within the time-delay-range (Min_{del}: Max_{del}), obtaining a measure of the correlation strength between devices' data for different time delays from the settable device. The delay time for which there is a higher correlation strength between devices' data is considered the synchronisation time ($Sync_{del}$), which has to be added to or subtracted from the settable device original-time.

2.4 Data management and statistical analyses

2.4.1 Reliability and performance of the method

To assess the confidence and performance of the proposed synchronization method, we used the data issued from case studies 1 and 2, which represent two sources of data of different nature and for which the true time delay between devices was known; for the purpose of this objective, in the case study 2 we compared only two pairs of temperature sensors: 5-15 m and 5-35 m - therefore omitting other temperature pairs as they yield similar results. Specifically, we searched for the synchronisation time (i.e., the true time delay between devices) using different options for the parameters SDR, SWS and Treatment. By Treatment, we refer to the type of statistic-handling of data from the sampling-windows selected every certain random time intervals, existing the levels "None" (i.e., when we use the raw data without calculating any statistics), "Mean", "RMS" and "aVeDBA". Besides, we also compared the performance of the method for two different temporal autocorrelation levels in data: high and low. To do so, we sub-sampled two shorter temporal windows of the same duration, one in which the data temporal autocorrelation was the highest and another one in which was the lowest we could find. In the case study 1 (acceleration data), the periods were of 90-min or 120-min for Maria's and Antonio's cases, respectively. In the case study 2 (temperature data), we used 2-months time periods. To check how the SDR, SWS or Treatment might affect the method performance, we switched between levels of one of those aspects (e.g., SWS) while maintaining the rest of the aspects (i.e., SDR and Treatment) at a fixed pre-established level (see Table 2.1). In the Treatment case, we compared two different formulas (or treatments) to calculate activity: the abovementioned one was the already mentioned RMS (Eq. 1), and the other was the “average Vectorial Dyinamic Body Acceleration” (aVeDBA, Eq. 2):

$$aVeDBA = \frac{\sum_{i=1}^{n} \sqrt{\left(X_{dyn}^2 + Y_{dyn}^2 + Z_{dyn}^2\right)}}{n} \, (Eq. 2),$$

which is analogous to RMS, but in this case the square root is applied to each sum of the squares of the measurements on the three axes and then added together, whereas in the case of RMS the square root is applied to the addition of all the sums of the squares of the individual measurements. As for temperatures, we compared the treatments "Mean" and "None", which refers to calculating an average temperature for the whole sampling-window or to use raw temperatures, respectively. When comparing the synchronisation performance as a function of the temporal autocorrelation, we used the default level for both the configurable parameters (i.e., SWS, SDR and Treatment). Since the sampling-delay is random, we performed 30 simulations for each configuration of the parameters and for each specific time delay of the settable device to get an average measure of the correlation strength between device data with its associated standard deviation (SD). Correlation strength was measured and compared using the Spearman rank correlation coefficient (r_S), considering a critical α-value of 0.05. Spearman coefficient was preferred to the Pearson product-moment correlation coefficient due to its non-parametric nature (based on ranks), which is appropriate for non-linear relationships and avoids the need for data to meet any assumption (Schober *et al*. 2018). Here the TDR and TDRes parameters were always constant for each case study (Table 2.1).

Table 2.1. Summary of the synchronizations performed throughout the study. The type of data (i.e., accelerations or temperatures), pair of devices synchronised ("Pairs"), sampling delay range ("SDR"), sampling-window size ("SWS"), selected-data treatment ("Treatment"), autocorrelation level (“Autocorrelation”), time delay range ("TDR") and time delay resolution ("TDRes") is indicated. Those pairs of devices with which we assessed the performance of the configurable parameters "SDR", "SWS" and "Treatment", as well as the importance of the temporal autocorrelation are highlighted in grey, indicating the distinct setups used. The default level of a configurable parameter when comparing across-levels synchronisation performance for another configurable parameter is indicated in bold. The temporal autocorrelation when comparing the among-levels sync performance for SDR, SWS and Treatment was the data's own ("inherent").

Data type	Pairs	SDR	SWS	Treatment	Autocorrelation	TDR	TDRes
Acc.	Antonio	**20s/80s**, 120s/180s, 270s/330s	1s, **15s**, 60s	**RMS**, VeDBA	High, Low	+50s / +100s	0.1s
	Maria	**20s/80s**, 120s/180s, 270s/330s	1s, **15s**, 60s	**RMS**, VeDBA	High, Low	-40s / +10s	0.1s
	H4	120s/360s	57s	RMS	Inherent	-60s / +60s	1s
	H5	120s/360s	57s	RMS	Inherent	-60s / +60s	1s
	H7	120s/360s	57s	RMS	Inherent	-60s / +60s	1s
	H8	120s/360s	57s	RMS	Inherent	-60s / +60s	1s
	D5	120s/360s	57s	RMS	Inherent	-60s / +60s	1s
	D8	120s/360s	57s	RMS	Inherent	-60s / +60s	1s
Temp.	5m-15m	**6h/18h**, 42h/54h, 90h/102h	**5h**, 24h, 36h	Mean, **None**	High, Low	-48h / +48h	1h
	5m-25m	6h/12h	5h	None	Inherent	-48h / +48h	1h
	5m-35m	**6h/18h**, 42h/54h, 90h/102h	**5h**, 24h, 36h	Mean, **None**	High, Low	-48h / +48h	1h
	15m-25m	6h/12h	5h	None	Inherent	-48h / +48h	1h
	15m-35m	6h/12h	5h	None	Inherent	-48h / +48h	1h
	25m-35m	6h/12h	5h	None	Inherent	-48h / +48h	1h

2.4.2 Method utility

To show the appropriateness of the proposed synchronization method, we used the remaining pairs of data-loggers of the case study 2 not synchronized previously (i.e., 5-25m, 15-25 m, 15-35 m, 25-35 m), and the six pairs of data-loggers of the case study 3 (“H4”, “H5”, “H7”, “H8”, “D5” and “D8”). The synchronisation of the temperature data-loggers was performed using the more appropriate SDR, SWS and Treatment (specified in Table 2.1) according to what we observed in the previous analysis. In the acceleration biologger and acceleration transmitter case, the synchronisation was accomplished using the acceleration transmitter as the reference device and the acceleration biologger as the settable one. We used complete-time periods to synchronise all the pairs of devices since the temporal autocorrelation was low enough to find the true time delay between them.

All statistical analyses were performed using the R software environment version 3.6.2 (R Core Team 2019). Spearman rank correlation coefficient (r_s) was calculated using the function ‘cor( )’ from the base R software. The autocorrelation quantification was done using the function ‘autocorrelation( )’ from the ‘colorednoise’ package version 1.1.0, which uses a lag of one timestep and algebraic formulation is based on Ruokolainen *et al*. (2009).

3. Results

We obtained 228 584 and 647 784 acceleration records for each pair of acceleration biologgers attached to “María” and “Antonio”, respectively, which corresponds to a period of approximately 5 hours for “Maria” and 15 hours for “Antonio”. Also, we got 8760 hours of simultaneous temperature data per depth level in the MPA of Cabo de Palos-Islas Hormigas between 1st June 2016 and 31st May 2017. Finally, we collected around 59 hours of simultaneous activity data from the acceleration transmitter and the acceleration biologger for the six wild dusky groupers tagged (Table 2.3, Fig. 2.3). In general terms, the temporal autocorrelation was appreciably lower for acceleration than for temperature data, although there were considerable differences among individuals or depths (Table 2.2, Fig. 2.4). Also, the difference in temporal autocorrelation between the "High" and "Low" levels was substantially larger for the acceleration data than for the temperature data (Table 2.2). Importantly, data temporal autocorrelation varied depending on the SWS, although this effect differed between temperatures and accelerations data. For temperatures, different SWS did not change temporal autocorrelation (Table 2.2). In the case of the accelerations, it depended on the SDR: while the temporal autocorrelation decreased as the SWS increased when using continuous data (SDR of 0s:0s), it increased when using intermittent data (SDR of 20s:80s) (Table 2.2).

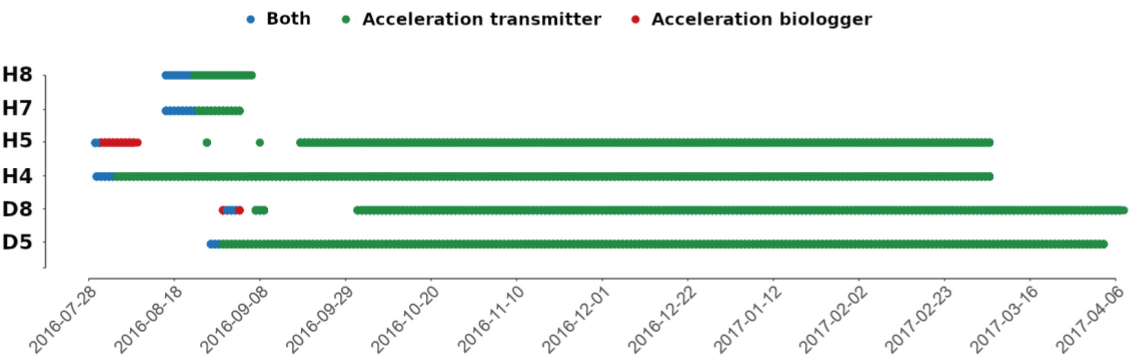

Figure 2.3. Recording timeline per individual for both the acceleration transmitter and the acceleration biologger simultaneously (blue), only for the acceleration transmitter (green) and only for the acceleration biologger (red). Note the large temporal scale of the study (~ 8 months).

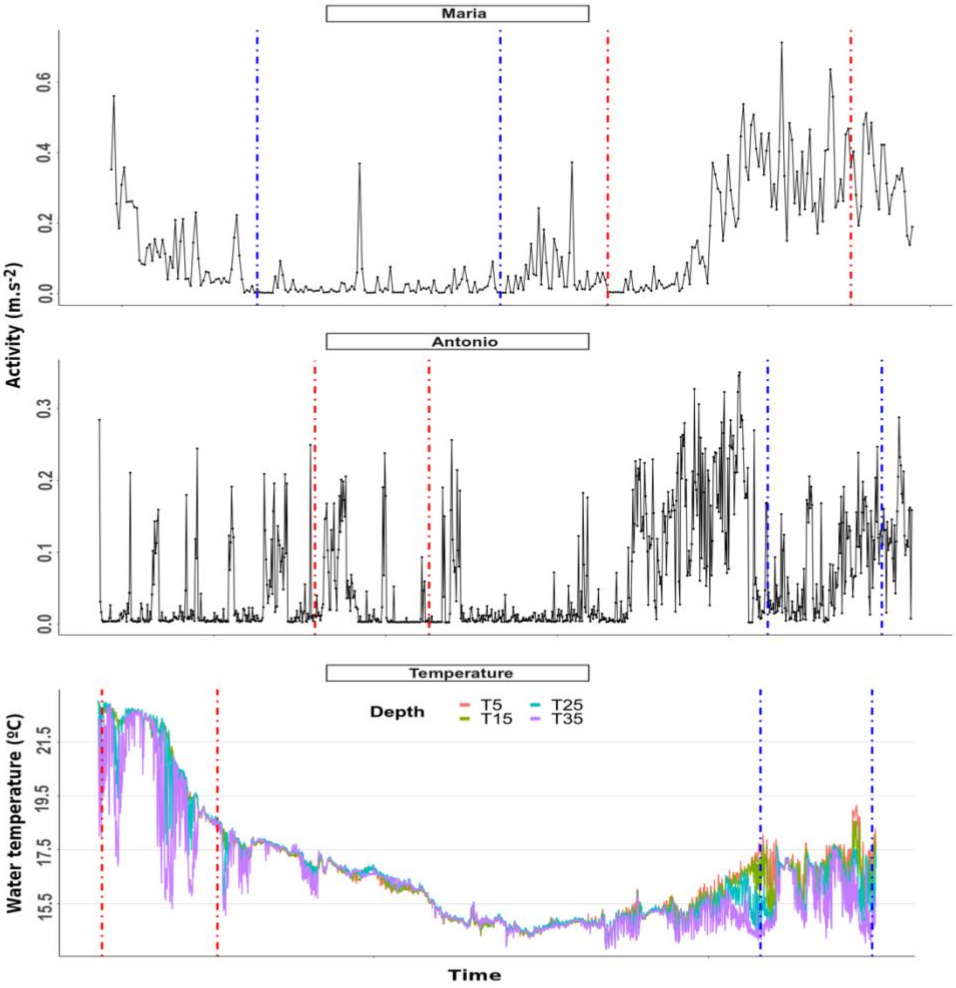

Figure 2.4. Plot showing Maria's and Antonio's activity as well as the water temperature (in degrees Celsius) at different depths (5, 15, 25 and 35 meters) in the marine reserve of Cabo de Palos over time. It is also shown the selected periods with high and low temporal autocorrelation (red and blue vertical dashed lines, respectively) compared to the whole period. Activity is estimated at sampling-window sizes of 57-s.

Table 2.2. Temporal autocorrelation degree of data for the whole period (Autoc$_{WP}$), for the short periods in which we assessed the effect of a high or low autocorrelation degree (Autoc$_{SP}$) and for data using different SWS (Autoc$_{SWS}$). Both Autoc$_{WP}$ and Autoc$_{SP}$ were calculated using the default settings (see Table 1) for the configurable parameters. Autoc$_{SP}$ and Autoc$_{SWS}$ were calculated only for those pair of electronic tags for which we assessed the performance of the configurable parameters (case study 1 and 2). For María and Antonio cases, Autoc$_{SWS}$ was calculated both using an SDR of 0s:0s (i.e., continuous data) or using the default one (i.e., 20s:80s) for interpretative reasons. For the temperatures case, Autoc$_{SWS}$ was calculated only for SDR of 6h:18h since a SDR of 0h:0h correspond to Autoc$_{WP}$.

Data type	**Ind/ Depth**	**Autoc$_{WP}$**	**Autoc$_{SP}$**		**Autoc$_{SWS}$**		
			High	Low	1s (0s:0s/20s:80s) 5h (6h:18h)	15s (0s:0s/20s:80s) 24h (6h:18h)	60s (0s:0s/20s:80s) 36h (6h:18h)
Acc	D5	0.496	-	-	-	-	-
	D8	0.831	-	-	-	-	-
	H4	0.548	-	-	-	-	-
	H5	0.718	-	-	-	-	-
	H7	0.560	-	-	-	-	-
	H8	0.386	-	-	-	-	-
	Maria	0.767	0.743	0.441	0.849/0.474	0.767/0.579	0.778/0.646
	Antonio	0.787	0.836	0.537	0.844/0.413	0.787/0.646	0.797/0.655
Temp	T5m	0.999	0.996	0.931	0.998	0.999	0.999
	T15m	0.997	0.996	0.906	0.995	0.996	0.997
	T25m	0.988	0.970	0.925	0.980	0.986	0.986
	T35m	0.959	0.950	0.927	0.949	0.956	0.956

Table 2.3. Summary of the number of acoustic signal detections and acceleration records from the acceleration transmitter and the acceleration biologger per dusky grouper, respectively. In brackets is indicated the approximated number of hours it corresponds considering that the acceleration transmitter was set to estimate activity for SWS of 57s and the acceleration biologger recorded acceleration at 12 Hz.

ID	**Acceleration transmitter**	**Acceleration biologger**
D5	659 (10 h)	3,240,000 (75 h)
D8	208 (3 h)	4,659,747 (105 h)
H4	589 (9 h)	5,232,623 (120 h)
H5	48 (0,76 h)	10,191,870 (235 h)
H7	1,566 (24 h)	8,536,903 (195 h)
H8	550 (9 h)	7,202,794 (165 h)

The discrepancies in the synchronisation time as a function of the type of configurable parameter (i.e., SWS, SDR and Treatment) used did not exceed 0.3 and 1.6 seconds for Maria's and Antonio’s scenarios, respectively (Table 2.4). Concerning the temperature data, in five out of the six pairs of temperature data-loggers, the time delay identified was always of 0 hours (Fig. 2.5, Fig. 2.6, Fig. 2.7). Only for one pair of temperature data-loggers (“T5m-T35m”) the proposed synchronisation method failed to find the synchronisation time independently of the setting for the configurable parameters (Table 2.4, Fig. 2.5). In this case, the synchronisation method only estimated the correct time delay between the data-loggers when the data was reduced to a shorter period (two months), intended to compare the effect of a “High” or “Low” temporal autocorrelation in the synchronisation performance (Table 2.4, Fig. 2.5).

In all cases in which the synchronisation method succeeded in finding the synchronisation time, there was a point of maximum correlation strength between device data from which the correlation dropped clearly towards both sides (Fig. 2.5, Fig. 2.6, Fig. 2.7). However, this pattern was no present for "T5m-T35m", in which the correlation strength between device data kept close to its maximum for a wide time delays range (Fig. 2.2). Lastly, it was also observed a generalized higher variability (i.e., SD) regarding the correlation strength between devices data as the time delay used was further from the true time delay, especially for the acceleration data (Fig. 2.5).

Configurable parameters (i.e., SDR, SWS and Treatment) showed to be of minor importance in the synchronisation process. In the case of "T5m-T35m", none of their settings allowed finding the true time delay (Table 2.4, Fig. 2.5). On the contrary, for the pair "T5-T15 m", the correct synchronisation time was found regardless of the setting-type. Only in the pairs of accelerometers ("Maria" and "Antonio") the configuration type caused the selected synchronisation time to oscillate, though minimally (Table 2.4). In the SDR and Treatment case, the oscillations in synchronisation time never exceeded 0.2 s and 0.3 s for "Antonio" and "María", respectively (Table 2.4). For the SWS, the oscillations were somewhat larger (1.6 s) only for "Antonio" (Table 2.4). In this case, the larger the SWS, the greater the distance between the selected synchronisation time and the true one

(90 s) (Table 2.4). For "Maria", the selected synchronisation time was always the same regardless of the SWS. However, for both "Antonio" and "Maria", a clear improvement in the synchronisation performance was observed as the SWS decreased since the difference in correlation strength between the true synchronisation time and the remaining time delays increased (Table 2.4).

Table 2.4. Results of the performance comparison among-levels of each configurable parameter (i.e., ASD, SWS and Treatment) and two temporal autocorrelation degrees (“High” and “Low”) when synchronising. The default level of a configurable parameter when comparing among-levels synchronisation performance for another configurable parameter is indicated in bold. As for the level of autocorrelation selected when assessing the other configurable parameters, this was the inherent one (neither high nor low) since we do not select shorter time windows than the original ones. For each synchronisation, the estimated true time-delay or "synchronisation time" ($Sync_{time}$) and the difference between the maximum and minimum correlation strength value ($Corr_{range}$), as a measure of synchronisation time identification easiness, are shown.

Parameter	Settings	Performance	Antonio	María	T5m-T15m	T5m-T35m
SDR	*270s/330s* *90h/102h*	$Sync_{time}$	88.9s	-29.3s	0h	5h
		$Corr_{range}$	0.209	0.238	0.018	0.033
	120s/180s *42h/54h*	$Sync_{time}$	89.0s	-29.6s	0h	-46h
		$Corr_{range}$	0.209	0.230	0.018	0.028
	20s/80s ***6h/18h***	$Sync_{time}$	89.1s	-29.4s	0h	-47h
		$Corr_{range}$	0.207	0.237	0.018	0.028
SWS	*60s/36h*	$Sync_{time}$	87.6s	-29.4s	0h	-46h
		$Corr_{range}$	0.099	0.132	0.019	0.027
	***15s**/24h*	$Sync_{time}$	89.1s	-29.4s	0h	-25h
		$Corr_{range}$	0.207	0.237	0.018	0.028
	*1s/**5h***	$Sync_{time}$	89.2s	-29.4s	0h	-47h
		$Corr_{range}$	0.223	0.245	0.018	0.028
Treatment	***RMS/none***	$Sync_{time}$	89.1s	-29.4s	0h	-47h
		$Corr_{range}$	0.207	0.237	0.018	0.028
	VeDBA/Mean	$Sync_{time}$	89.1s	-29.4s	0h	-25h
		$Corr_{range}$	0.197	0.231	0.016	0.028
Autocorrelation	*High*	$Sync_{time}$	88.4s	-29.6s	0h	0h
		$Corr_{range}$	0.224	0.236	0.098	0.050
	Low	$Sync_{time}$	90.3s	-29.3s	0h	0h
		$Corr_{range}$	0.372	0.571	0.380	0.138

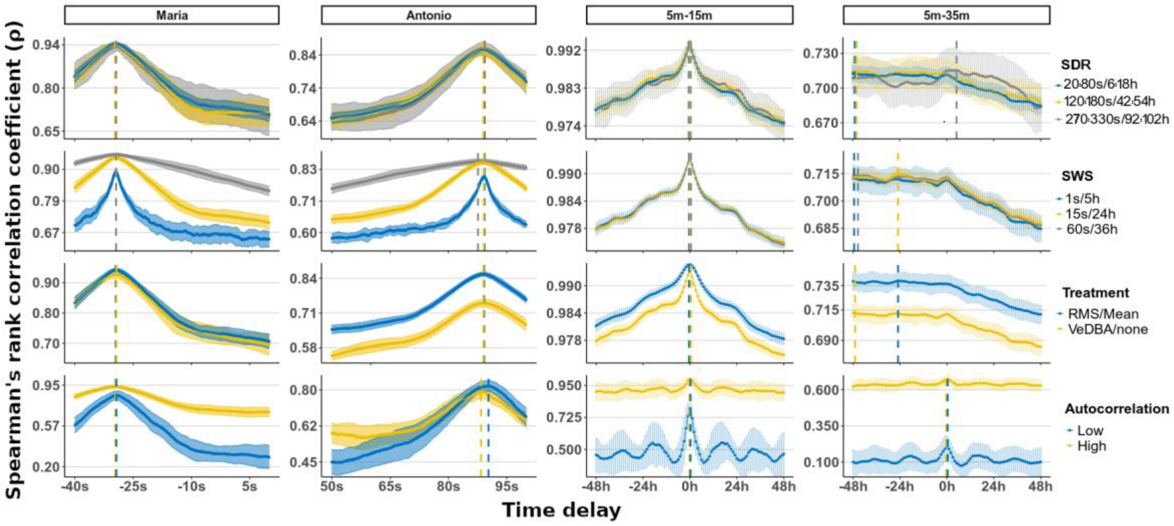

Figure 2.5. Set of plots showing the synchronization performance using different levels of the configurable parameters (i.e., SDR, SWS and Treatment). The default level for the configurable parameters is indicated in Table 2. The synchronisation performance is also shown as a function of the Autocorrelation degree, which can be "High" or "Low" and is relative to the data's inherent temporal autocorrelation for the complete time series. The temporal autocorrelation values are indicated in Table 2.2. It is important to note that the Y-axis scale changes between the different graphs.

However, more important than any of the configurable parameters of the synchronization method was an aspect related to the nature of the data: its temporal autocorrelation degree. Both for accelerations and temperatures data, low temporal autocorrelations improved our ability for identifying the right time delay, increasing the correlation strength differences sharply among the true time delay and the rest of time delays (Table 2.4, Fig. 2.5). This was especially evident with the pair of devices "T5-T35 m", for which the reduction in temporal autocorrelation through shortening the original time period (one year) to two months was enough for finding the true time delay (Table 2.4, Fig. 2.5). Here, in line with the rest of cases in which the temporal autocorrelation was assessed ("Maria", "Antonio" and "T5-T15 m"), the higher correlation strength difference between the synchronisation time and the rest of time delays was with a low temporal autocorrelation in data (Table 2.4).

In the case of the six pairs of acceleration transmitter/acceleration biologger to be synchronised on wild dusky groupers, the synchronisation time ranged considerably among pairs, with values between -5 s ("H7") and +40 s ("H5"). In four out of the six pairs ("H7", "H8", "H4" and "D5") there was a clear time delay point from which the correlation strength between device data decreases smoothly towards both sides (Fig. 2.6). For "D8" and "H5", although there was also a clear decline in the correlation strength between device data as we moved from the selected synchronisation time, it was sawtooth-shaped, with some quite high correlation strength values 2-3 seconds away from it (Fig. 2.6).

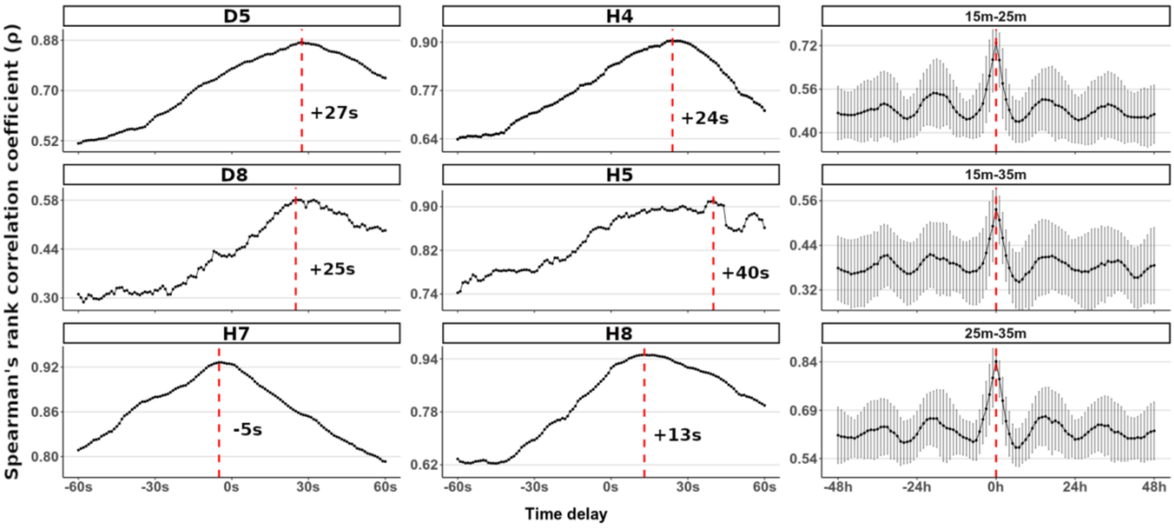

Figure 2.6. Set of plots showing the true time-delay or “synchronisation time” (vertical red dashed lines) between different pairs of electronic tags according to the method proposed. The first two columns refer to the case study 3 (an acceleration transmitter and an acceleration biologger in six dusky groupers) while the last column to the pairs of temperature data-loggers “15m-25m”, “15m-25m” and “25m-35m”. For the case study 3, it was also showed the synchronisation time values for interpretability reasons.

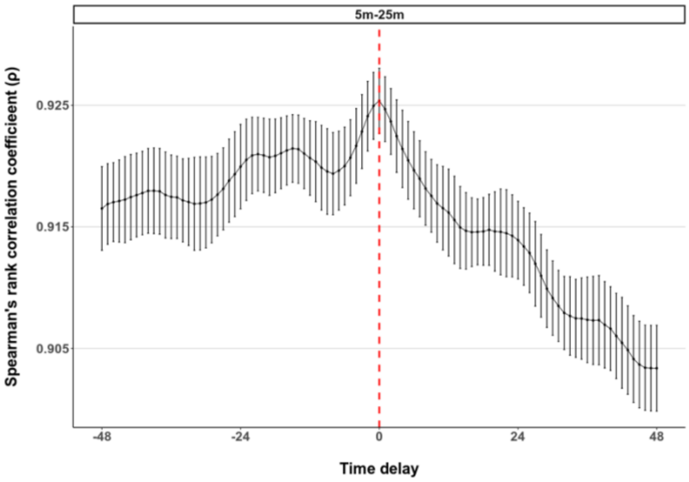

Figure 2.7. Plots showing the true time-delay or “synchronisation time” (vertical red dashed lines) between the pairs of data-loggers “T5m-T25m” according to the method proposed.

4. Discussion

In this study, through empirical data of different nature (accelerations and temperatures), we were able to demonstrate that data recorded simultaneously from different sensors can be synchronised whenever such data are correlated to some extent. Our initial assumption was that if data issued from two devices are inherently correlated by their nature, it is most likely that their maximum correlation strength occurs when they are fully synchronised. Here, using eight different pair of devices for which we knew the time delay between them, we demonstrated the validity of that assumption. In 100% of the cases, we found the true time delay or "synchronisation time" using a procedure based on the change of the time of one device to check when the correlation strength between device data is maximum. We believe that the fact that we could not know the true time delay between the pairs of acceleration biologgers on the scale of tenths of a second did not prevent us from demonstrating the validity of the assumption behind the proposed synchronisation method, which was the primary aim of this study. Besides, regardless of the type of devices we would have used, it is not possible to set the time with tenths of a second precision, as it is usually done manually.

Clearly, the data attribute that most affected the synchronisation performance was their temporal autocorrelation degree, as this triggers high correlation strengths between devices data even when they are desynchronised. This is supported by the observed larger range between the maximum and minimum correlation strength for the pairs of accelerometers than for those of temperatures, which had a much higher temporal autocorrelation. The minor effect of the SWS, SDR, and Treatment in the accuracy of the synchronisation process suggests that the setting for the so-called “configurable parameters” does not alter the robustness of the assumptions behind the proposed procedure. As mentioned in results, only the SWS showed a slight effect on the synchronisation performance of acceleration data, which improved as we reduced the SWS. This is likely related to the SWS effect on the data temporal autocorrelation. In the pairs of accelerometers, small SWS (e.g., 1 s) reduced the temporal autocorrelation compared to long SWS (e.g., 60 s). But importantly, this effect occurred only when using intermittent instead of continuous data (i.e., SDR distinct from 0). That is, the temporal autocorrelation was higher at small SWS when using acceleration data at continuous (i.e., without sub-sampling). This can be explained given that the smaller the SWS, the more likely that consecutive activity values are similar. Importantly, this result highlights the advantages of using intermittent instead of continuous data in certain circumstances, such as when using accelerations data, as it can helps to reduce temporal autocorrelation of the data. In the case of temperatures, there were no differences in temporal autocorrelation for different SWS, which is likely the reason why the synchronisation performance did not change depending on the SWS for temperatures. The fact that at SWS of 60 s increased the deviation considerably from the true time delay for the "Antonio" and not for "Maria" is possibly evidence of the influence of other data aspects when synchronising, such as the natural correlation strength between device data. In Maria's case, it was higher than for Antonio and could be why only in Antonio case the synchronisation performance was slightly affected. However, the "T5-T35 m" case shows that even with relatively low correlation values (maximum mean ρ value of 0.21), the true time delay can be correctly calculated. The reason why we were able to accurately calculate it with "T5-T35 m" and not with "Antonio", even though in the former case the conditions in terms of temporal autocorrelation and correlation strength between device data were worse, is possibly related to the data-processing type. While for the pairs of temperature sensors the correlation measure between data was using raw data from the SWS, in the case of the accelerometers the data from the SWS were transformed to single activity values using the RMS formula. This fact possibly makes the SWS effect larger for acceleration data and would be supported by the fact that SWS was only shown to be a relevant aspect in the performance of the synchronisation procedure for acceleration data.

Sharp differences in the synchronisation time among individuals for case study 3 are evidence of the complexity of the clocks-time drift issue. In any study, two strategies can be used to synchronise pairs of devices. The first and preferable one would be to synchronise them simultaneously just before the start of the experiment. The second one would be to synchronise the pairs of devices at different times but always using a reference time (e.g., https://time.is). However, in field experiments such as the one developed in the case study 3, the first scenario is practically unaffordable since the deployment of the array of receivers and the capture and tagging of fish usually take several days or even weeks at best. Thus, if we consider that the time drift of any clock starts when it is configured, regardless of whether it is switched off after that, it is likely to find that the pairs of devices desynchronised at the time of starting to record. This occurs because the clock-time drift changes in magnitude and even in direction among clocks since, among others, they are subject to different temperature and pressure conditions and might be of different ages. Besides, those devices attached to aquatic animals such as the dusky grouper undergo unpredictable and sharp changes in pressure and temperature over time, making it impossible to accurately calculate the time-clocks drift as we do not know what conditions they have been subjected to. Evidence of that is the clock-time drift for "H7" (-5s), which is of opposite sign to the rest (positive), highlighting that clock-time drift patterns might change unpredictably even when always using the same models of device (in our case, VR2W and X16-mini). Furthermore, the amount of data is a key aspect to consider: few data for the pairs of devices increases the likelihood that correlation of devices data is high even with some time delay between them, especially if the temporal autocorrelation is high, causing higher risks of error in the estimation of synchronisation time; conversely, too many data associated to long time periods may limit the synchronisation capability if we calculate a single time delay value for the whole time period, as the drift changes over time. That is, the time delay between a pair of devices might be substantially different at the beginning and end of a study if it lasts for a long period relative to the clock drift, making a single time delay with which to correct the entire time series of one of the devices inadequate. All this implies a trade-off between a minimum amount of data to increase the probability of selecting the correct synchronisation time and a maximum time period in which to search for the synchronisation time according to the theoretical clocks drift. As an example of this, in our case study 3 the individuals with the least amount of data ("H5" and "D8") showed the most uncertainty in the selected synchronisation time. However, these issues also depend on the temporal resolution of the data and the magnitude of the clocks-time drift. For example, if we consider that an average time-delay between sensors could be 1 s per day, in a year, there will be a time drift between sensors of 6 minutes, which is not a problem when operating at 1-hour time resolutions (case study 2) but it is indeed at 0.1-second ones (case study 3).

Solutions to the problem of the time synchronisation of sensors operating simultaneously are scarce in the literature, and those available are complex to apply, case-specific or need some consideration before data-acquisition. Here we have shown a useful and general approach to synchronising devices after data-acquisition. Arguably, it is useful for any data if the requirements about simultaneous data are met and there is a certain correlation between the devices data. The proposed solution is certainly useful in situations where the devices were not initially synchronised or to check they were correctly synchronised. Before applying the proposed method, the resolution of data (i.e., 1 data per second, per hour, per day, etc.) and the clock-time drift scale should be considered to decide the most appropriate time periods in which synchronise pair of devices. Also, to achieve the most accurate synchronisation possible, the amount of data should be as much as possible, and the temporal autocorrelation should be minimised at the lowest with strategies such as increasing the SDR or selecting specific time periods. The R-code shared to perform the synchronisation is open, and further research to improve the method is encourage.

Chapter III

Can we use acoustic telemetry data to infer aquatic animal activity? A field test using the top predator fish *Epinephelus marginatus* (Lowe, 1834) as model species

Can we use acoustic telemetry data to infer aquatic animal activity? A field test using the top predator fish *Epinephelus marginatus* (Lowe, 1834) as model species

ABSTRACT

Acoustic telemetry has become one of the leading technologies to study behaviour in aquatic animals. Activity is among the most studied traits given its crucial role for the investigation of animal ecology. Different activity proxies have been used in the context of acoustic telemetry studies (e.g., number of detections, depth range, distance travelled or acceleration), but they present some constraints that condition the quality of data that can be obtained. Until now, the attention paid to those constraints has primarily focused on the factors affecting the ability of propagation (and therefore, of reception) of the acoustic signal. In this study, we combined high frequency tri-axial accelerometer data with acoustic telemetry data to evaluate and compare the performance of different variables commonly used as activity proxies in the context of acoustic telemetry. Using the top-predator dusky grouper *Epinephelus marginatus* (Lowe 1834) as a model species, we evaluated possible consequences of using internal acceleration transmitters, the consequences of averaging their activity into time bins, the existence of sampling-bias and the performance of three common but non-validated activity proxies derived from acoustic telemetry (number of detections, and depth range). Our results suggest that acceleration transmitters provide the only reliable activity estimates, particularly for low activity levels, rejecting the widely accepted but none-validated assumption of the number of detections or the depth range as proper activity proxies. Finally, we identify some possible issues when using acceleration transmitters such as high-activity sensitivity loss or individual-dependent sampling bias, providing advice and perspectives for future studies of fish activity involving acoustic telemetry. Therefore, in the light of these results, we conclude that results about animals activity patterns drawn thought acoustic telemetry without using acceleration data as the activity proxy should be interpreted with caution, encouraging the review of those in future research using appropriate methods for inferring activity.

1. Introduction

The study of animal behaviour has gained relevance over the years mainly due to its importance for biodiversity conservation and ecosystem functioning (e.g., Sutherland 1998, Réale *et al*. 2007, Nagelkerken & Munday 2016). However, studying animal behaviour is complex and particularly challenging in the aquatic environment. In the last decades, acoustic telemetry became one of the leading technologies used to describe different aspects of the behaviour of aquatic animals given its advantages over other technologies (Hussey *et al*. 2015). Briefly, it consists of using acoustic transmitters, animal-borne devices that emit acoustic pulses, and acoustic receivers configured to hear and decode those acoustic signals. Each acoustic signal successfully heard by a receiver is typically referred to as a 'detection'. The main advantage of acoustic telemetry over other technologies used for the study of aquatic animals behaviour is that it allows obtaining data from benthic animals without the need of recapturing the individuals. Moreover, the limited ability of acoustic signals to propagate in the aquatic environment (i.e., hundreds of meters), together with the possibility to incorporate different sensors into the acoustic transmitters (e.g., temperature, pressure or acceleration), have led to a wide variety of alternatives for the study of behaviour (Hussey *et al*. 2015): from the simple characterization of presence/absence patterns (e.g., Kessel *et al*. 2016), to more complex ones determining the drivers of animals' behaviours or their cyclicality (e.g., Alós *et al*. 2017, Kneebone *et al*. 2018), personality issues (e.g., Villegas-Ríos *et al*. 2017) or even the study of fine-scale movements (e.g., Itakura *et al*. 2017, Becker *et al*. 2020, Leander *et al*. 2020). All this leads acoustic telemetry to play a central role nowadays in the study and understanding of aquatic animals behaviour, which is pivotal for designing adequate management measures that ensure the sustainability of human activities (Brownscombe *et al*. 2019).

The study of activity is one of the traits most widely addressed in animal behaviour research. Immelmann & Beer (1989) defined activity, in an ethological sense, as "an individual animal's general, or specific, movement". The broad nature of this term allows activity to be inferred from numerous approaches using acoustic telemetry. One approach uses displacement data as a proxy for activity, associating the distances travelled (either horizontal or vertical) with the activity level (e.g., Moland *et al*. 2011, McLean *et al*. 2014, Giacalone *et al*. 2015, Itakura *et al*. 2017, Gandra *et al*. 2018). Another approach consists in counting the number of detections per time bin, which is routinely interpreted as an indicator of the activity level (e.g., Chateau & Wantiez 2007, Payne *et al*. 2010, Béguer-Pon *et al*. 2015). However, acoustic telemetry presents important technological limitations that might bias the data obtained and, thus, the activity patterns interpretation (Cooke *et al*. 2016, Brownscombe *et al*. 2019). For instance, it is entirely possible that tracked individuals are indeed very active at a specific depth or at a smaller spatial scale than the resolution capabilities of the receivers array designs (Cooke *et al*. 2016). Also, the number of detections might be more influenced by environmental factors (Payne *et al*. 2010, Gjelland *et al*. 2013, Huveneers *et al*. 2016) or the position of the animal (changing its audibility) (Swadling *et al*. 2020) rather than by its activity level. Nevertheless, the lack of clear evidence against the use of these variables as proxies for activity has led them to be widely used, highlighting an important knowledge gap that is urgent to address.

The (relatively) recent incorporation of acceleration sensors (i.e., accelerometers) in the field of behavioural ecology has supposed a breakthrough in understanding animal behaviour, including activity patterns, given the close relationship between animals acceleration and energetic costs (Wilson *et al*. 2019). Acoustic telemetry companies started to offer acoustic transmitters equipped with accelerometers around 2008, providing a new horizon of possibilities in studying aquatic animal behaviour (Murchie *et al*. 2011, Cooke *et al*. 2016). Accelerometers measure raw accelerations, commonly in three orthogonal axes (X, Y, Z), at customised sampling frequencies expressed typically in ‘s^{-1}’ or hertz (Hz). This type of data has proven to be very useful to describe and quantify animal behaviours such as activity or body posture (e.g., Lyons *et al*. 2013, Ciancio *et al*. 2016, Beltramino *et al*. 2019), having a great potential to be combined with other types of data (e.g., McClintock *et al*. 2013, 2017). However, acceleration data derived from acoustic transmitters have some particularities which should be considered when interpreting them (Murchie *et al*. 2011, Cooke *et al*. 2016). The first one is associated with the resolution of acoustic transmitters, which can transmit 256 different activity values. This forces a trade-off between a wide activity range (defined by the minimum and maximum measurable activity) with low resolution (i.e., large differences among transmissible activity values) or a short one with high resolution. Secondly, the high energy cost of transmitting acoustic signals, combined with limited batteries lifetime and the need of transmitting the data, forces acoustic accelerometers to record activity intermittently, at low sampling frequencies and using low acceleration ranges compared to the standards provided by traditional (non-acoustic) accelerometers (Cooke *et al*. 2016). Besides, other considerations arise when using acoustic telemetry derived acceleration data. For instance, although the internal (i.e., intragastric or in the coelomic cavity) placement of acoustic transmitters is widespread due to its advantages over the external attachment (Brownscombe *et al*. 2019), the consequences of bearing an accelerometer free inside the animal body remain unknown (Wilson *et al*. 2019). On other occasions, researchers are interested in the association of data obtained non-simultaneously by their acoustic transmitters (e.g., activity and depth), or they need to regularize temporally the data, binning them into broad time windows (Taylor *et al*. 2013, Udyawer *et al*. 2017, Runde *et al*. 2020). This, however, beyond causing the loss of temporal resolution, might also trigger the loss of accuracy in activity estimations, as the number and type of activity samples obtained could be influenced by the habitat in which the animal stays (Swadling *et al*. 2020). For example, if a species tends to rest (i.e., low activity) during the night in a shelter (i.e., acoustic signals non-audible by receivers) but taking occasional excursions (i.e., audible periods) with high activity peaks, while during the day it tends to stay outside its shelter (i.e., audible periods) with higher activities compared to the night, we could conclude that activity was higher at night due to a biased sample. Thus, while the incorporation of accelerometers in acoustic telemetry is a major advance, it should not be forgotten that there are some inherent limitations of acoustic telemetry that may limit the ability of accelerometers onboard acoustic transmitters to estimate activity, which makes further research essential to understanding the performance of this type of data.

This study aimed to assess the performance of different acoustic telemetry data typically used to infer activity patterns in aquatic animals. To do so, we equipped a top predatory fish [dusky groupers, *Epinephelus marginatus* (Lowe 1834)] with internal acoustic transmitters that incorporate an accelerometer (henceforth “acceleration transmitter”) and external acceleration data-loggers (henceforth “acceleration biologger”) that records acceleration continuously at higher resolutions (12 Hz vs 5 Hz). Specifically, we compared data from both devices assuming that acceleration biologgers provide the most reliable activity estimation given their body-attachment mode and lower technological restrictions. To our knowledge, this is the first study in which the performance of different acoustic telemetry data, including accelerations, are evaluated in natural (field) conditions using an independent tri-axial acceleration biologger, thereby improving the fish behave realism and allowing to assess the consequences of some of the already mentioned acoustic telemetry constraints. The specific goals of this paper were: (1) to assess the performance of acoustic accelerometers for measuring activity in aquatic animals, 2) to evaluate the effects of the time bin size when binning activity from acoustic accelerometers, (3) to assess potential biases in activity data acquisition with acoustic telemetry; and (4) to assess the performance of three common but non-validated activity proxies derived from acoustic telemetry.

2. Materials & Methods

2.1 Species and study area

The present work was accomplished on a top predator fish species, the dusky grouper (*E. marginatus*) at the Cabo de Palos – Islas Hormigas Marine Fishery Reserve (CPIH-MFR) (Murcia, Spain, Western Mediterranean) between early August 2016 and early April 2017. This Marine Protected Area (MPA) harbours a series of seamounts lined up in the west-east direction, two of which reach the surface and result in the Hormiga and Hormigón islets (Fig. 3.1). These seamounts are rocky reefs typically surrounded by a low-slope sandy bottom of detritic nature. In the shallower areas, these bottoms are partially covered by *Posidonia oceanica* meadows (Pérez-Ruzafa *et al*. 1991, Charton & Pérez-Ruzafa 1998). The dusky grouper is a demersal species strongly associated with rocky substrates, where it usually seeks small cavities for shelter (Barreiros & Santos 1998, Harmelin & Harmelin-Vivien 1999). This deep-rooted behaviour has led to the fact that on many occasions, the number of detections has been associated with the activity (e.g., Hackradt 2012, Koeck *et al*. 2014, Afonso *et al*. 2016). This species is ideal for developing fine-scale behavioural studies using acoustic telemetry due to its sedentary behaviour and high site fidelity, particularly in its adult stage (Pastor *et al*.

2009, Koeck *et al*. 2014, Afonso *et al*. 2016). Besides, it promotes high interest in different fields (Condini *et al*. 2018), namely scientific (it is a keystone species due to its crucial role controlling marine food webs from top to bottom), economic (it is a high esteemed target both for the commercial and recreational sectors), and for conservation (the IUCN Red List of Threatened Species categorized the dusky grouper as a Vulnerable Species since 2018 [Pollard *et al*. 2018]).

2.2 Passive acoustic monitoring and acceleration recording

Two arrays of acoustic receivers (model VR2W, Vemco, Inc.), were deployed, one around the Hormigón island (HG) at depths between 18 and 51 m (n=13), and the other around the 'Bajo de Dentro' reef area (BD) between 18 and 46 m depth (n=10). The receivers were placed less than 100 meters apart between each other (Fig. 3.1) following existing recommendations for coral reef habitat studies (Welsh *et al*. 2012). This, along with the consideration that the detection range estimated for this area is 250 m (Hackradt 2012), maximized the probability for the acoustic signals to be recorded by the receivers whenever the tagged individuals were in those rocky reefs and outside the caves. For each detection, the receivers registered date and time, a unique ID for each fish, as well as either an acceleration or a pressure (depth) value.

We used two types of tri-axial accelerometers simultaneously to estimate activity in free-living fish: an acceleration transmitter onboard an acoustic transmitter (model V13AP, Vemco, Inc.) and an acceleration biologger (model X16-mini, GCDC, Inc). A total of 16 fish (8 around HG and 8 around BD) were equipped with an acoustic transmitter, which was placed internally and built-in both tri-axial acceleration and pressure (depth) sensors. Ten out of them (from H4 to H8 in HG and D4 to D8 in BD) were also equipped with an external acceleration biologger (model X16-mini, GCDC Inc.). Acceleration transmitters typically record acceleration at low frequency (5 or 10 Hz) and intermittently (they have to transmit the data), have a narrow acceleration range (<±4G) and provide a single activity value for wide but customisable sampling windows (15 s, 30 s, 50 s, etc.) which is typically calculated using the root mean square (RMS) formula (Eq. 1). This last occurs because acceleration transmitters can only transmit 256 different values, corresponding '0' to the minimum activity (normally 0 m s^{-2}) and '255' to the maximum one, which in the case of VEMCO tags can be set to 3.4 m s^{-2} or 4.9 m s^{-2}. It is important to note that the higher the maximum activity set for acceleration transmitters, the lower the activity resolution (i.e., the ability to distinguish between different activity levels). In contrast, acceleration biologgers record acceleration continuously (data is not transmitted but stored in an internal memory of the device) and at frequencies up to 800 Hz or higher, usually have wide acceleration ranges (>±15G), and most importantly, allow raw (i.e., at high resolution) data to be obtained. The main advantage of acceleration transmitters over acceleration biologgers is that they do not require the recapture of the individuals to obtain data, also allowing to know the approximate area where the individuals were due to the limited propagation capacity of the acoustic signals. Although the acoustic transmitters register and transmit data at random time intervals, acceleration recording starts immediately after the depth record transmission. This, added to the synchronisation method used (described in Chapter 2), allowed us to match data from the acceleration transmitters and the acceleration biologgers.

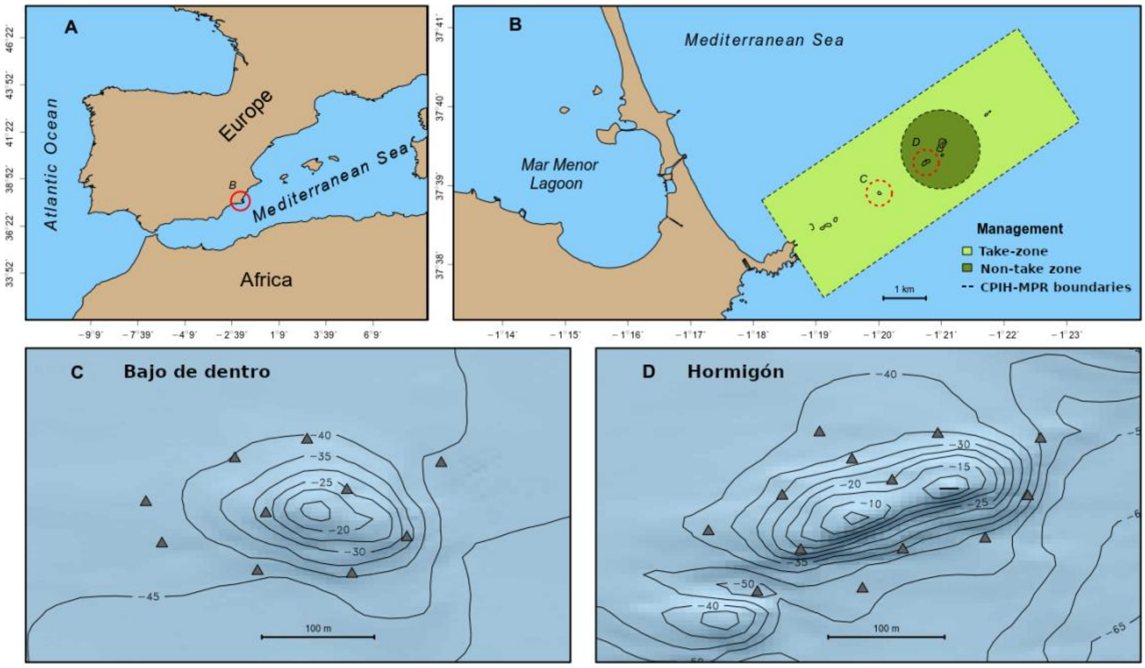

Figure 3.1. Maps illustrating a general overview of the situation of the Cabo de Palos - Islas Hormigas Marine Fishery Reserve (A), the location of the study sites (Bajo de Dentro [BD] and Hormigón [HG]) in the context of the CPIH-MFR (B), and the bathymetry as well as the acoustic receivers' network

design both in BD (C) and HG (D). Figure B also shows the spatial distribution of the two protection levels present in the MPA.

2.3 Fish handling, tagging, and recovering

We used artisanal baited traps to capture fish; for this purpose, we used traps made of wicker, built expressly to reproduce both a local type of fish traps (copied from a model recovered from an old picture) and a 'gambin'-type trap (once typical of the Balearic Islands), both specific models for the capture of groupers. All captured fish were adults, ranging in size between 50 and 92 cm total length and an estimated weight ranging 2-12 kg (Reñones *et al*. 2010). Once fish were on board, they were placed in a cooler with seawater regularly aerated and replaced. The swim bladder was deflated with a hollow needle prior to handling. Once stabilized, fish were moved to other cooler containing the anaesthetic solution (40 mg L^{-1}; 1-part clove oil emulsified in 9-parts ethanol). The anaesthesia procedure followed the ethical recommendations of Neiffer & Stamper (2009). Fish were maintained in the anaesthetic solution until loss of balance and lack of response when pressing the caudal peduncle. Once anaesthetized, they were placed on an ichthyometer for the surgery. The head was covered with a wet towel and cold seawater was run through the gills during the surgery. First, we made a 3-cm incision in the ventral cavity using a sterile surgical blade, to introduce the acceleration transmitter. After that, we sealed the incision using surgical glue to avoid the permeability of the wound. For 10 out of the 16 operated fish (range size = 54 to 89 cm), we also fixed the acceleration biologger externally close to the dorsal fin (Fig.3.2). The acceleration biologger was encapsulated with heat-shrink tubing following Ciancio *et al*. (2016), and attached to the animal with biodegradable monofilament surgical suture (Monomax 1 HR37, B. Braun Inc) and two sterile buttons. This type of attachment was performed to be sure that the devices would be released if we were not able to recapture the individuals, although it allowed some animals to hold them for up to two months after placement (Pereñíguez, J.M., pers. obs.). The total weight (in the air) of our devices was approximately 16 g and 13 g for the acceleration biologger and the acceleration transmitter respectively, and therefore, they never exceeded 1% of the body mass of individuals. Betadine and an antibiotic spray were spread on the manipulated areas. Both accelerometers were always placed keeping the same orientation in all individuals. After a short recovery, fish were released in the same area where they had been captured. Fish behaviour was checked by a diver for at least 10 minutes from their released at the surface to the moment in which they leaned on the bottom or they hid in some cave. The external accelerometers were recovered through fish recapture (using baited traps) between 14 and 45 days after releasing, liberating individuals in the same area where they were captured, with no abnormal behaviour signs. Handling and tagging procedures followed recommendations described by Wagner *et al*. (2011) and were performed by authorized personnel. We also had the pertinent permits both for working in the protected area and for ethical aspects related to animal welfare.

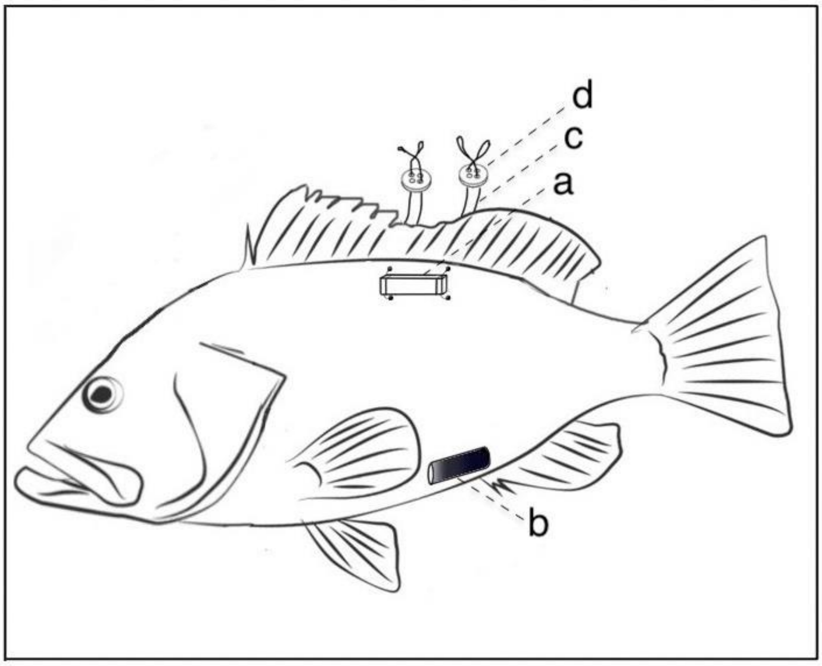

Figure 3.2. Schematic representation of the position in which accelerometers were placed in the wild dusky groupers. a: acceleration biologger (model X16-mini, GCDC Inc.); b: acceleration transmitter (model V13AP, VEMCO Inc.); c: biodegradable monofilament surgical suture; d: sterile button. The illustration design was based on Rai (2016).

2.4 Device settings

Given the low overall activity of *E. marginatus* and following recommendations by VEMCO, acceleration transmitters were set to record tri-axial acceleration at a fixed sampling-window size (SWS) of 57 s and with a recording frequency of 5 Hz, calculating what we called “acoustic activity” (i.e., activity derived from the acceleration data obtained with the acoustic transmitter). Specifically, the acceleration transmitters calculate activity using the “root mean square” (RMS) (Cooke *et al*. 2016), which is calculated through the expression:

$$RMS = \frac{\sqrt{\sum_{i=1}^{n}\left(X_{dyn}^2 + Y_{dyn}^2 + Z_{dyn}^2\right)}}{SWS * \omega} \, (Eq. 1)$$

where X_{dyn}, Y_{dyn} and Z_{dyn} represent the dynamic acceleration (m s^{-2}) in the X, Y and Z axes, respectively; *SWS* represent the size of the sampling-window (in seconds) and ω, the recording frequency (Hz). The dynamic (given by fish movements) and static (given by the orientation of the devices and gravity) components of the raw acceleration recorded by the acceleration transmitters were separated by using the low pass filter “Butterworth” with a 3.5 s cut-off (Vemco, pers. comm.).

The acceleration transmitters (onboard the acoustic transmitters) were set to send activity and pressure (i.e., depth) records alternatively with an average random delay among consecutive emissions of 120 s (range: 60 s to 180 s). They have an acceleration range of ± 29.4 m s^{-2} (i.e., ± 3G) and provided activity values which range between 0.0 m s^{-2} and 4.91 m s^{-2}, implying a resolution of 0.019 m s^{-2}. The pressure sensor from the acoustic transmitters was set to the “full-scale” pressure option of 68 m, with an accuracy and resolution of 3.4 m and 0.3 m, respectively. Lastly, acoustic transmitters were set to have an output power of 153 dB, having an expected battery life according to their settings of 214 days (info provided by VEMCO). To account for differences in clock drift among receivers, they were time-synchronised using VUE software provided by Vemco.

The acceleration biologger have an acceleration range of ± 156.8 m s^{-2} (i.e., ± 16G). They were set to record acceleration at a frequency of 12 Hz, having an expected battery life of around 8 days (used with a 250mAh battery). The raw accelerations were separated into their static and dynamic components by using a 3.5 s running mean, following suggestions by Vemco (pers. comm). Here, we estimated the average vector of the dynamic body acceleration (aVeDBA, Eq. 2) as a proxy for activity in goals 2, 3 and 4 (see below) for two main reasons: 1) we observed it is much less affected than RMS by changes in the time bin size (see Appendix C) and 2) it has been extensively tested in the scientific literature as an activity proxy (e.g., Qasem *et al*. 2012, Wright *et al*. 2014, Thiem *et al*. 2015, Metcalfe *et al*. 2016, Wilson *et al*. 2019). Goal 1 was the only case in which we used the formula RMS to calculate activity from the acceleration biologgers to discard that the differences observed in activity estimates between the acceleration transmitter and the acceleration biologger were due the use of different proxies for activity.

$$aVeDBA = \frac{\sum_{i=1}^{n}\sqrt{\left(X_{dyn}^{2} + Y_{dyn}^{2} + Z_{dyn}^{2}\right)}}{SWS * \omega} \, (Eq.2)$$

2.5 Data analyses

In the next subsections (2.5.1 to 2.5.4), we explain the rationale and the procedure for achieving each of the goals. Finally, in the subsection 2.5.5 we specify the general statistical and modelling tools used. As the number of detections can be highly affected by the environmental conditions (e.g., Mathies *et al*. 2014, Huveneers *et al*. 2016), we excluded from the analyses those time bins with standardised detection frequencies (SDF) values below 0.8, following Payne *et al*. (2010). In other words, we excluded those hours in which receivers ability to detect the acoustic signals had been reduced by at least 20% compared to average conditions in the area.

2.5.1 Goal 1: Performance of the internal acoustic transmitter accelerometers

Under the assumption that the acceleration biologgers obtain more accurate activity estimates from our model fish species (*E. marginatus*) than those from the acceleration transmitters, given the type of attachment and characteristics of the former ones, we compared activity from both devices. Specifically, using linear mixed-effect models (LME), we evaluated the relationship between the data series obtained by both devices. Models were constructed by considering activity estimations made by the acceleration biologger ($\mathrm{Act}_{\mathrm{bio}}$) as the dependent variable and those from the acceleration transmitters (Act_{trans}) as the explanatory variable. Data were log-transformed to meet model assumptions. To test for potential non-linear relationships between data obtained from both devices, we also tested polynomial forms of degrees 1 to 5, and estimated their explained variances.

2.5.2 Goal 2: Effects of binning acoustic activity into time bins

This subsection explored the predictive power of acceleration transmitters on acceleration biologgers activity estimates (Act_{bio}) at different time bin sizes. Specifically, we considered the effects of both the average activity values from the acceleration transmitters (Act_{trans}) and the number of activity detections (n_{trans}), which is indicative of the fraction of the time for which acceleration has been sampled and thus it could be expected higher accuracies as 'n_{trans}' increases. Besides, it could be expected some relationship between Act_{bio} and n_{trans} given the known behaviour of *E. marginatus*, which tend to be static when they are not audible by the receivers (hidden in their refuges). Therefore, we examined the linear-relationship between Act_{bio}, Act_{trans} and n_{trans} at time bin sizes of 6 min (n=3960), 15 min (n=2027), 30 min (n=1079), 60 min (n=561) and 120 min (n=284). We used LME models, considering the acceleration biologger activity estimation (Act_{bio}) as the dependent variable, and Act_{trans}, n_{trans} and their interaction, as numerical variables. All the variables were log-transformed to meet model assumptions.

2.5.3 Goal 3: Assessment of potential sampling-bias in data acquisition

The spatial behaviour of tagged animals might bias activity data-acquisition, especially in species with hiding tendencies such as dusky grouper: if their activity changes among habitats in which the acoustic signal detection probability also differ (e.g., open spaces vs. caves), we may obtain a non-representative or biased sample of activities. Thus, to test possible sampling-bias for acceleration transmitters, we used acceleration data from acceleration biologgers and calculated activity (aVeDBA formula) at SWS of 20 s, categorising the sampling-windows in which there was no detection from the acoustic transmitters (either with activity or depth data) as 'reference windows' and those in which there was as 'detection windows'.

The SWS (i.e., 20 s) was chosen based on a trade-off between a SWS small enough to represent the activity properly around the detection time and large enough to do not increase the sample-imbalance between both types of windows substantially. We used the unpaired two-sample Wilcoxon test for individual fish to test for differences in activity values between the reference (considered as the true central tendency) and detection windows. First, we applied a two-sided test and, in the case of rejecting the null hypothesis of equality in central tendencies between both types of windows, we applied a one-tailed test to check if the central tendency of the activity values was higher or lower for the detection windows than for the reference ones. We also calculated the effect size (*r*), a useful measure to assess the magnitude of the differences between window-types (Tomczak & Tomczak 2014).

2.5.4 Goal 4: Assessment of the performance of four common proxies for activity

In acoustic telemetry is common to use non-validated variables such as the number of detections or the depth-range per time bin as activity proxies. Thus, we assessed the performance of the variables ‘number of unique detections’ ($\text{Unique}_{\text{det}}$), ‘number of total detections’ ($\text{Total}_{\text{det}}$) and ‘depth-range’ ($\text{Depth}_{\text{range}}$) as activity proxies, all of them derived from the acoustic transmitters. Specifically, $\text{Total}_{\text{det}}$ and $\text{Unique}_{\text{det}}$ represent the number of detections of each acoustic transmitter ID considering or not time-unique transmissions in multiple receivers, respectively, and $\text{Depth}_{\text{range}}$ the depth change of each acoustic transmitter ID, being all of them calculated at time bins of one hour, a common time bin size in the scientific literature (see for example Payne *et al*. 2010 or Koeck *et al*. 2014). We analysed data at two different time scales: at short-term scale (in the range of a few days), for which data from the acceleration biologgers was available (n=583), and at long-term scale (about 6 months), for which only data from the acceleration transmitter were available (n=12,065) (Fig. 3.3). In the short-scale scenario, we used hourly activity estimates from the acceleration biologger (Act_{bio}) as the reference activity (response variable) and fitted different LME models containing only one of the explanatory variables at a time: $\text{Unique}_{\text{det}}$, $\text{Total}_{\text{det}}$ or $\text{Depth}_{\text{range}}$. On the long-scale scenario, we proceeded similarly but using hourly activity averages from the acceleration transmitters ($\text{Act}_{\text{trans}}$) as the reference activity (response variable). The performance of the three activity proxies was evaluated by comparison with the corresponding null LME (where only an intercept was included). The explained variance for each model was also estimated for interpretation purposes.

Finally, to assess the potential implications of the limitations of acceleration transmitters for studying aquatic animals activity patterns, we compared results between the acceleration biologger and the acceleration transmitter concerning the effect of a widely used environmental variable: the 'Time of day' (ToD) (e.g., Villegas-Ríos *et al*. 2013, Brownscombe *et al*. 2019). Specifically, we used raw activity data from the acceleration transmitter (Act_{trans}) (i.e., intermittent activity estimates at SWS of 57 s) and activity estimates from the acceleration biologger (Act_{bio}) (i.e., continuous activity estimates) using SWS of 57 s. The idea behind this analysis was to explore potential effects of using different proxies for activity rather than to describe plausible activity patterns in *E. marginatus*. To do so, we used the unpaired two-sample Wilcoxon test for each fish separately given the importance of individual trends (see for example, Alós *et al*. [2017] or Beltramino *et al*. [2019]). The procedure was the same used for solving Goal 3. Here, 'ToD' was categorical and had two levels: 'day' and 'night'. For this analysis, data from the individual H5 was discarded since there was no available data during 'night'.

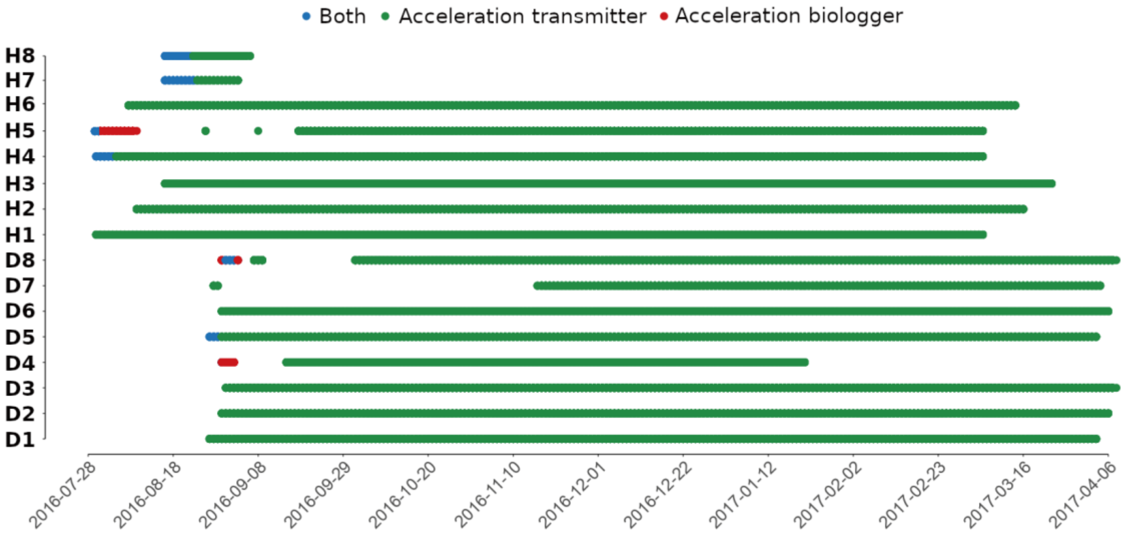

Figure 3.3. Recording timeline per individual for both the acceleration transmitters and the acceleration biologgers simultaneously (blue), only for the acceleration transmitter (green) and only for the acceleration biologger (red). Note the large temporal scale of the study (~ 8 months).

2.5.5 General statistical analyses and modelling tools

All statistical analyses were performed with R software v.3.6.2 (R Core Team). The appropriateness of the inclusion of the fish identity as a random factor (either as an intercept or both as an intercept and slope) in LME was tested comparing its fit against a generalized least squares (*gls*) model without a random factor. LME and GLS models were performed using the R package 'nlme' (Pinheiro *et al*. 2018). The random terms in LMEs were evaluated by fitting the models using restricted maximum likelihood (REML), while the fixed terms were tested using the maximum likelihood (ML) method. Final models were fitted by REML (Zuur *et al*. 2009). All models were visually validated by checking normality and homoscedasticity of residuals (Zuur *et al*. 2010). We used Akaike's information criterion (AIC) to run for model selection, choosing the one with the lowest AIC value (Burnham & Anderson 2002). The variances explained by the models were calculated using two goodness-of-fit measures (Nakagawa & Schielzeth 2013) with the MuMIn R package (Barton 2020): marginal goodness-of-fit (r^2_{m}), which indicates the variance explained by the fixed factors, and conditional goodness-of-fit (r^2_{c}), which shows the variance accounted for both the fixed and the random terms. The non-parametric unpaired two-sample Wilcoxon test and the effect size were performed using the functions 'wilcox.test' and 'wilcox_effsize', available in the ´base´ package of the R software (R Core Team 2019).

3. Results

Data of the acceleration transmitters and the acceleration biologgers distributed heterogeneously among individuals (Fig. 3.3 and Table 3.3). We obtained 2,059,831 unique detections from the acoustic transmitters implanted in the 16 dusky groupers tagged in the present study. The ratio depth:activity data from the acoustic transmitters was approximately 1:1 (n_{depth}=1,029,143 and n_{activity}=1,030,688). Seven out of ten fish that had acceleration biologgers attached to their bodies were recovered, four from HG and three from BD. One fish (D4) was completely out of range of the acoustic receivers while the acceleration biologger was recording; hence, this fish was excluded from the analyses. Other fish (H5) left the study area eight hours after its release and it returned a few weeks later, being watched in the meantime in another reef, Piles II, located 2 km away. A total of 39,063,937 acceleration records (850 hours) were obtained for the six individuals tagged with the acceleration biologger and included in the analysis (Table 3.1). Activity data from the acceleration biologgers and the acceleration transmitters were strongly right-skewed, and rarely (0.11%) reached the maximum activity value configured for the acceleration transmitter (Fig. 3.4).

Table 3.1. Summary of the number of detections obtained for both the acceleration biologger and the acceleration transmitter while they were deployed simultaneously. The rounded total amount of recording time is shown in parentheses. Note that when the fish were not audible (hidden or outside from the receivers' detection range), we had no data on the acceleration transmitter.

ID	Acceleration transmitter	Acceleration biologger
D5	659 (10 h)	3,240,000 (75 h)
D8	208 (3 h)	4,659,747 (105 h)
H4	589 (9 h)	5,232,623 (120 h)
H5	48 (1 h)	10,191,870 (235 h)
H7	1,566 (24 h)	8,536,903 (195 h)
H8	550 (9 h)	7,202,794 (165 h)

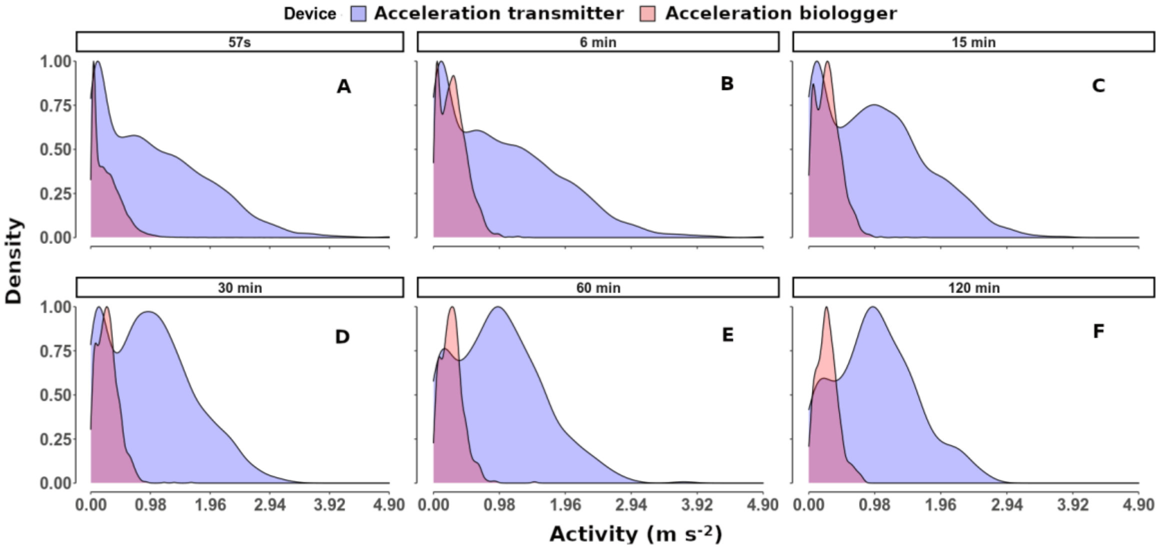

Figure 3.4. Kernel density plots showing the distribution of the activity values from the acceleration biologgers and the acceleration transmitters, at time-intervals of 57s (A), 6 minutes (B), 15 minutes (C), 30 minutes (D), 60 minutes (E) and 120 minutes (F). Note that the activity values from the acceleration transmitters at SWS of 57s implies they are raw activity values.

3.1 Goal 1: Performance of internal acoustic transmitter accelerometers

For most of the activity range recorded for *E. marginatus*, the RMS values obtained with the acceleration transmitters were larger than those produced with the acceleration biologgers (Fig. 3.5A). Acceleration transmitters explained 88.3 % of the total variance of the acceleration biologgers. Fish ID (Random component) accounted for an additional 5% (Table 3.2). The best relationship between the activity estimated by the acceleration biologger and the acceleration transmitter in a log scale involved a fourth-degree polynomial (Table 3.2). Although a fourth-degree model was chosen over a linear model (i.e., first-degree polynomial) because several activity values recorded by the acceleration transmitter clearly broke away from the linear trend (Fig. 3.5B), the linear model represented almost equally well (88.0 %) the relationship between activity estimates from the acceleration transmitter and the acceleration biologger. The slopes of the fitted curves in the logarithmic scale lost its linear trend at activity values of the acceleration transmitter equal to or lower than 0.058 m s^{-2} in its natural scale.

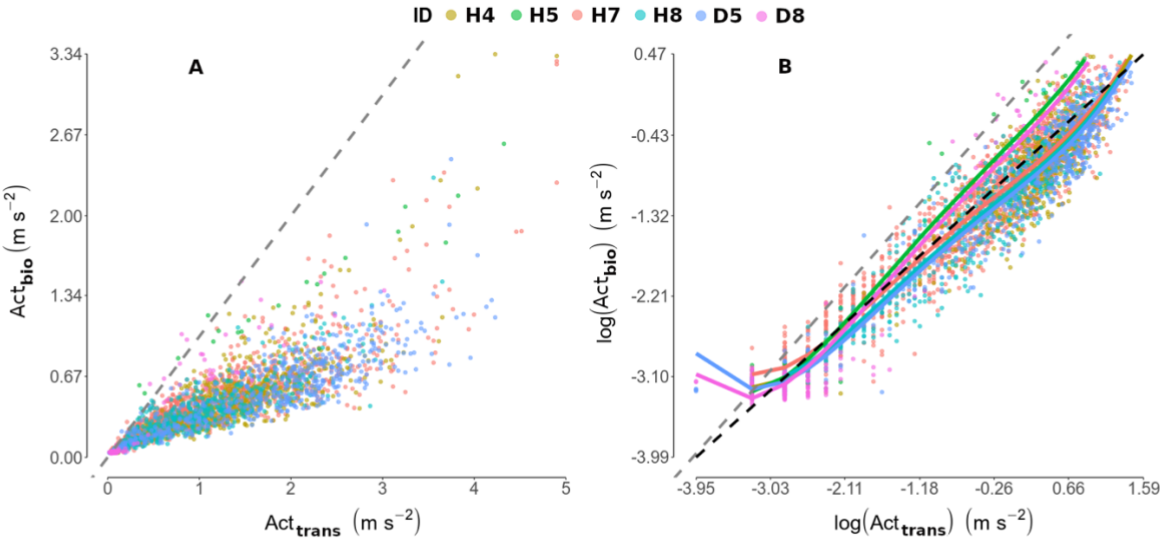

Figure 3.5. Scatterplots showing the relationship between the activity (in m s^{-2}) measured by the X16-mini ($Activity_{X16}$) and by the V13AP ($Activity_{V13AP}$) at 57s time-window sizes in their natural scale (A) and after log-transformation (B). Different individuals are identified with different colours. A solid regression line was added in the log-transformed data to show the fit of the selected LME model per individual. A grey dashed line was added in both panels to show a 1:1 relationship between variables as well as a black dashed line in the right panel to show the global fit of the linear (1st degree polynomial) model.

Table 3.2. Results of LMEs for the relationship between activity from the acceleration biologger (response) and activity from the acceleration transmitter (predictor) at SWS of 57s using different polynomial degrees. The fixed part structure of the models, coefficients estimation, explained variance by the fixed factors (r^2m) and by both the fixed and the random terms (r^2c), the degrees of freedom of the model (df) as well as the AIC of the models are shown. Significant coefficients (P-value ≤ 0.05) are marked with an asterisk. The selected model (lowest AIC) is highlighted in bold.

Model structure (fixed part)	**Coefficient**						**r^2m**	**r^2c**	**df**
	a	**b**	**c**	**d**	**e**	**f**			
$y = a + bx^1 + cx^2 + dx^3 + ex^4 + fx^5$	**-1.183***	**60.432***	**3.52***	**-1.201***	**3.363***	**0.538**	**0.8839**	**0.9363**	**10**
$y = a + bx^1 + cx^2 + dx^3 + ex^4$	-1.182*	60.445*	3.486*	-1.161*	3.361*		0.8835	0.9363	9
$y = a + bx^1 + cx^2 + dx^3$	-1.185*	60.404*	3.476*	-1.129*			0.8810	0.9336	8
$y = a + bx^1 + cx^2$	-1.187*	60.343*	3.315*				0.8830	0.9330	7
$y = a + bx^1$	-0.812*	0.805*					0.8803	0.9309	6

3.2 Goal 2: Effects of binning acoustic activity into time bins

Generally, both devices showed a decrease in the proportion of activity values close-to-zero as the time bin size increased (Fig. 3.4). For models predicting activity from the acceleration biologger (‘Act_{bio}’) at different time bin sizes, the model ranked first had different fixed structures depending on the time bin size (Table 3.3), but always including the predictors ‘$\text{Act}_{\text{trans}}$’ and ‘$n_{\text{trans}}$’. For the smallest bin sizes (6 and 15 minutes) only the predictors ‘$\text{Act}_{\text{trans}}$’ and ‘$\text{Act}_{\text{trans}} \times n_{\text{trans}}$’ were selected, while for the largest bin sizes (30, 60 and 120 minutes) the variable ‘n_{trans}’ was also included (Table 3.3). Overall, the predictive power of the fixed part of the models enlarged moderately as the time bin size increased, while it decreased for the random part (which included a random intercept and slope for each individual) (Fig. 3.6). For example, the fixed component of the model chosen for 6-min time bins explained 74% of the total variance, while the random component accounted for an additional 5% (Table 3.3). In the other extreme, for the case of 120-min time bins, the fixed component explained 82% of the total variance, while the random component accounted for an additional 1%. The relative influence of fish identity remained between 5% and 6% for the time-windows of 6 min and 15 min, respectively, but dropped markedly with larger time-windows: 3%, 1% and 1% for time-windows of 30 min, 60 min and 120 min, respectively.

The consideration of the variable ‘number of detections’ in any of its forms (either ‘n_{trans}’ and/or ‘$Act_{trans} \times n_{trans}$’) in the models allowed an increase of the predictive power of the fixed component. It was subtle for time bins of 6, 15 and 30 minutes (between 0.7% and 3.9%), and larger (rounding 10%) for those of 60 and 120 minutes. Finally, we observed an activity under-estimation tendency from the acceleration transmitter at low activity values compared with the acceleration biologger (Fig. 3.6). This trend was not captured by the linear models and vanished as the time bin size increased.

Table 3.3. Results of the LMEs assessing the effect of the time-interval (‘6 min’, ‘15 min’, ‘30 min’, ‘60 min’ and ‘120 min’) and the number of detections (n_{det}) in the relationship between activity from the acceleration biologger (Act_{bio}) and activity from the acceleration transmitter (Act_{trans}). The fixed part structure of the models, the coefficients’ estimate, the variance explained by the fixed factors (r^2m) and by both fixed and random terms (r^2c), the degrees of freedom of the models, as well as the AIC of the models, are shown. Significant coefficients ($p \leq .05$) are marked with an asterisk. The model selected (lowest AIC) by time-interval is highlighted in bold.

Time bin (min)	**Model structure (fixed part)**	**Coefficient**				**r²m**	**r²c**	**df**	**AIC**
		Interc.	**Act_{trans}**	**n_{det}**	**Act_{trans}:n_{det}**				
6	$Act_{bio} \sim Act_{trans}$	-1.239*	0.570*	-	-	0.7225	0.7614	6	4,449.0
	$Act_{bio} \sim Act_{trans} + n_{det}$	-1.230*	0.571*	-0.051*	-	0.7227	0.7616	7	4,451.1
	$Act_{bio} \sim Act_{trans} + Act_{trans}$:$n_{det}$	**-1.238***	**0.554***	-	**0.108***	**0.7277**	**0.7648**	**7**	**4,412.9**
	$Act_{bio} \sim Act_{trans} + n_{det} + Act_{trans}$:$n_{det}$	-1.238*	0.554*	-0.003	0.107*	0.7276	0.7647	8	4,420.7
15	$Act_{bio} \sim Act_{trans}$	-1.290*	0.548*	-	-	0.7152	0.7743	6	1,851.5
	$Act_{bio} \sim Act_{trans} + n_{det}$	-1.263*	0.550*	-0.034	-	0.7146	0.7750	7	1,856.5
	$Act_{bio} \sim Act_{trans} + Act_{trans}$:$n_{det}$	**-1.287***	**0.471***	-	**0.121***	**0.7407**	**0.7859**	**7**	**1,787.5**
	$Act_{bio} \sim Act_{trans} + n_{det} + Act_{trans}$:$n_{det}$	-1.322*	0.460*	0.043*	0.1345*	0.7442	0.7861	8	1,791.2
30	$Act_{bio} \sim Act_{trans}$	-1.271*	0.581*	-	-	0.7569	0.7960	6	829.8
	$Act_{bio} \sim Act_{trans} + n_{det}$	-1.327*	0.576*	0.037*	-	0.7566	0.7956	7	835.1
	$Act_{bio} \sim Act_{trans} + Act_{trans}$:$n_{det}$	-1.268*	0.476*	-	0.091*	0,776	0.8050	7	805.0
	$Act_{bio} \sim Act_{trans} + n_{det} + Act_{trans}$:$n_{det}$	**-1.454***	**0.414***	**0.125***	**0.130***	**0.7862**	**0.8077**	**8**	**789.5**
60	$Act_{bio} \sim Act_{trans}$	-1.292*	0.580*	-	-	0.7325	0.7599	6	466.0
	$Act_{bio} \sim Act_{trans} + n_{det}$	-1.342*	0.576*	0.025	-	0.7336	0.7592	7	472.6
	$Act_{bio} \sim Act_{trans} + Act_{trans}$:$n_{det}$	-1.284*	0.287*	-	0.167*	0.7741	0.7997	**7**	406.6
	$Act_{bio} \sim Act_{trans} + n_{det} + Act_{trans}$:$n_{det}$	**-1.752***	**0.123***	**0.225***	**0.244***	**0.8059**	**0.8142**	**8**	**367.3**
120	$Act_{bio} \sim Act_{trans}$	-1.297*	0.619*	-	-	0.7423	0.7635	6	224.7
	$Act_{bio} \sim Act_{trans} + n_{det}$	-1.823*	0.613*	0.194*	-	0.7831	0.7856	7	199.9
	$Act_{bio} \sim Act_{trans} + Act_{trans}$:$n_{det}$	-1.269*	0.143	-	0.190*	0.7758	0.8070	7	206.6
	$Act_{bio} \sim Act_{trans} + n_{det} + Act_{trans}$:$n_{det}$	**-1.979***	**0.011**	**0.249***	**0.228***	**0.8211**	**0.8293**	**8**	**157.8**

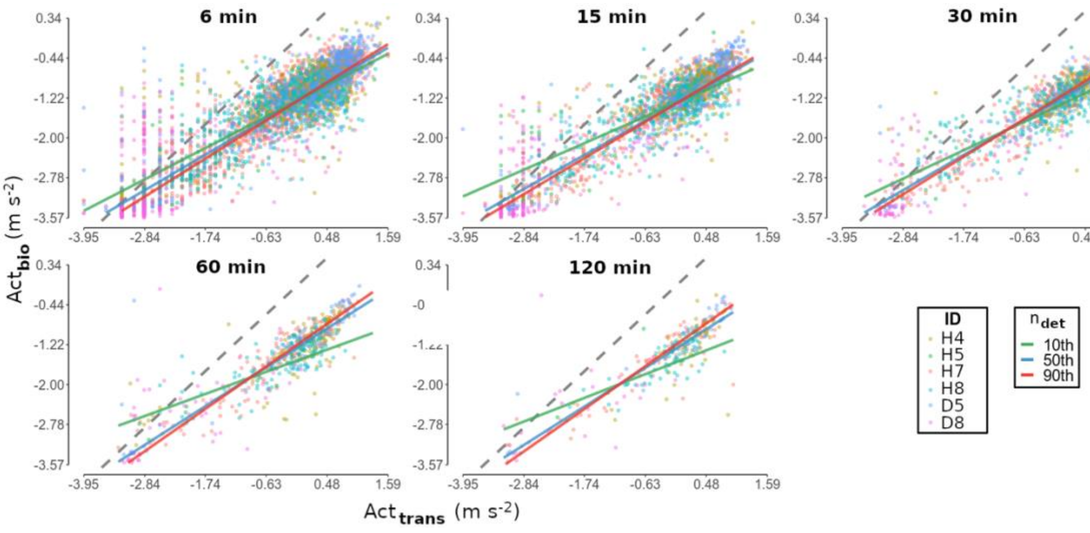

Figure 3.6. Scatterplots showing the relationship between activity from the acceleration biologgers (Act_{bio}) and averaged activity from the acceleration transmitters (Act_{trans}) by time interval. The continuous lines represent the fit of the fixed part of the selected model for three fixed number of activity detections (n_{det}): the 10th (green), 50th (blue) and 90th (red) percentile. The grey dashed line represents a 1:1 fit. In each time interval, we used the model that incorporated the fixed structure selected through the lowest AIC (Table 3.3).

3.3 Goal 3: Assessment of potential sampling-bias in data acquisition

The median activity was higher in most fish for the ‘detection’ window than for the 'reference' one (Table 3.4). We detected significant differences in activity between ‘reference’ and ‘detection’ windows in four out of the six tagged fish (Table 3.4, Fig. 3.7). Three of those fish had been tagged in HG, while the fourth one in BD. In all the individuals in which we found significant differences in activity between both types of windows (H7, H4, H8 and D8) the central tendency in the ‘detection’ windows was significantly higher than in the ‘reference’ ones ($P<0.05$), suggesting that in those individuals there was a trend of being more detected when they were more active. However, the effect size varied sharply among individuals (differences by up to one order of magnitude) and was, overall, small (i.e., effect size [*r*] <0.3, Tomczak & Tomczak 2014) for all the four individuals (Table 3.4).

Table 3.4. Descriptive statistics and results of the unpaired two-samples Wilcoxon test (UWT) for differences in activity between “reference” and “detection” windows. $Act_{Ref.W}$ and $Act_{Det.W}$: median activity for ‘reference’ and ‘detection’ windows, respectively; PCM: percentage of change between windows’ medians (calculated as 100 × [$Act_{Det.W}$ - $Act_{Ref.W}$) / $Act_{Ref.W}$]); r: effect size; $P_{Two\text{-}sided}$ and $P_{One\text{-}tailed}$: P-values for both the “two-sided” and the “one-sided” UWT, $n_{Ref.W}$ and $n_{Det.W}$: sample-size for ‘reference’ and ‘detection’ windows, respectively. Significant results (p ≤ 0.05) are marked with an asterisk.

ID	$Act_{Ref.W}$	$Act_{Det.W}$	PCM	r	P-val$_{Two\ sided}$	P-val$_{One\ tailed}$	n_{Ref}
H4	0.250	0.353	41.6	0.106	<0.001*	<0.001*	18,704
H5	0.040	0.040	0.2	0.011	0.7061	0.353	1,096
H7	0.226	0.245	8.0	0.021	<0.001*	<0.001*	29,646
H8	0.167	0.224	33.9	0.057	<0.001*	<0.001*	26,050
D5	0.343	0.340	-0.8	0.001	0.9368	0.5316	11,396
D8	0.033	0.033	1.2	0.022	<0.05*	<0.05*	8,398

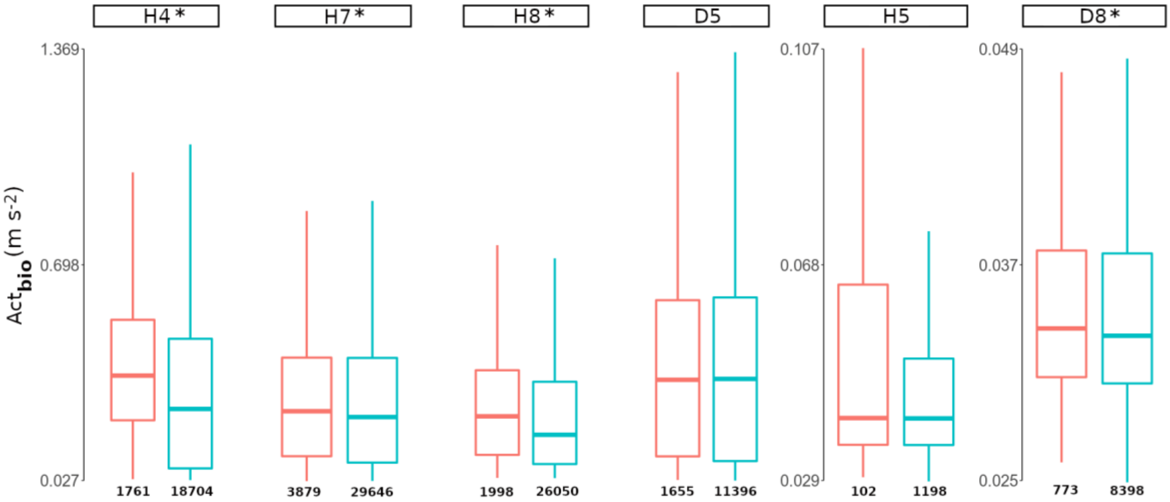

Figure 3.7. Boxplots of activity from the acceleration biologger (Act_{bio}) at sampling-window sizes of 20 s, per individual and window type (‘reference’ [blue] and ‘detection’ [red]). The sample-size is indicated below each bar). Individuals for which there were significant differences between window types are indicated with an asterisk. Note also we used three different ranges for the y-axis given the sharp among-individuals differences in the distribution of the activity values.

3.4 Goal 4: Performance of four common proxies for activity

When using short-term data, '$\text{Depth}_{\text{range}}$' was the only variable that showed some explanatory power (25%) of activity from the acceleration biologger (Table 3.5, Fig.3.8). '$\text{Unique}_{\text{det}}$' and '$\text{Total}_{\text{det}}$', on the contrary, had less statistical support than the null model, suggesting poor explanatory power (2% and 3%, respectively). These results were consistent with those found for the long-term analysis (Table 3.5, Fig. 3.9): '$\text{Depth}_{\text{range}}$' explained 18% of '$\text{Act}_{\text{bio}}$' variability while '$\text{Unique}_{\text{det}}$' and '$\text{Total}_{\text{det}}$' showed weak explanatory power (2% and 5%, respectively) (Table 3.5). For all the activity proxies and the temporal scales, the selected random structure was a random intercept and slope for each individual, with the differences among individuals (fish identity) contributing significantly to the accuracy of the predictions.

Table 3.5. Results of LMEs assessing the performance of the number of unique detections ($\text{Unique}_{\text{det}}$), the number of total detections ($\text{Total}_{\text{det}}$) and the depth range ($\text{Depth}_{\text{range}}$) as activity proxies for *E.marginatus* at a short and long temporal scale. The variance explained by the fixed factors (r^2m), by both fixed and random terms (r^2c), the degrees of freedom of the models, as well as the AIC of the models, are shown. The selected model (lowest AIC) is highlighted in bold. In both temporal scales, the different activity proxies are ordered from highest to lowest r^2m.

Temporal scale	Activity proxy	Model structure	r^2m	r^2c	df	AIC
Short	$\text{Depth}_{\text{range}}$	Act_{bio} ~ Intercept	0.0000	0.6719	5	894.2
		Act_{bio} ~ $\text{Depth}_{\text{range}}$	**0.2537**	**0.6481**	**6**	**890.4**
	$\text{Total}_{\text{det}}$	**Act_{bio} ~ Intercept**	**0.0000**	**0.4802**	**5**	**1,147.2**
		Act_{bio} ~ $\text{Total}_{\text{det}}$	0.0249	0.4613	6	1,151.6
	$\text{Unique}_{\text{det}}$	**Act_{bio} ~ Intercept**	**0.0000**	**0.4603**	**5**	**1,163.1**
		Act_{bio} ~ $\text{Unique}_{\text{det}}$	0.0194	0.4450	6	1,167.6
Long	$\text{Depth}_{\text{range}}$	$\text{Act}_{\text{trans}}$ ~ Intercept	0.0000	0.4320	5	122.686
		$\text{Act}_{\text{trans}}$ ~ $\text{Depth}_{\text{range}}$	**0.1796**	**0.2531**	**6**	**122.651**
	$\text{Total}_{\text{det}}$	$\text{Act}_{\text{trans}}$ ~ Intercept	0.0000	0.2634	5	135.099
		$\text{Act}_{\text{trans}}$ ~ $\text{Total}_{\text{det}}$	**0.0578**	**0.1674**	**6**	**135.085**
	$\text{Unique}_{\text{det}}$	$\text{Act}_{\text{trans}}$ ~ Intercept	0.0000	0.1389	5	138.323
		$\text{Act}_{\text{trans}}$ ~ $\text{Unique}_{\text{det}}$	**0.0206**	**0.1038**	**6**	**138.315**

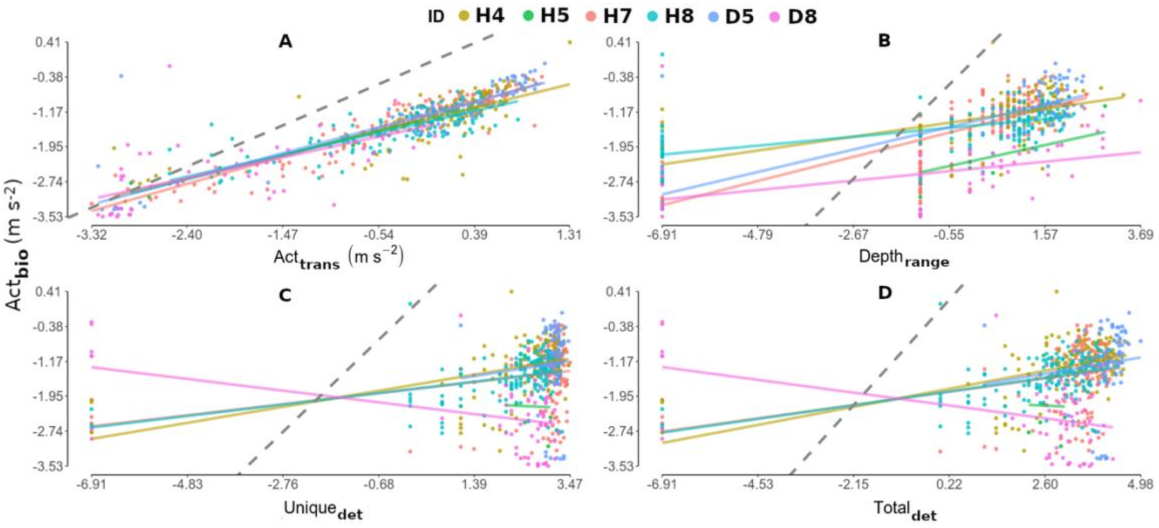

Figure 3.8. Scatterplots showing the relationship between activity estimates obtained with the acceleration biologgers (Act_{bio}) and the averaged activity from the acceleration transmitters (Act_{trans}; A), depth-range ($Depth_{range}$; b), number of unique detections ($Unique_{det}$; C) and number of total detections ($Total_{det}$; D) at the short-term (~ days). All the variables were calculated at one-hour time bin sizes and log-transformed. Different individuals are identified with different colours. In each panel, a solid regression line was added per individual (random factor) to show the fit of the corresponding LME and illustrate possible general trends.

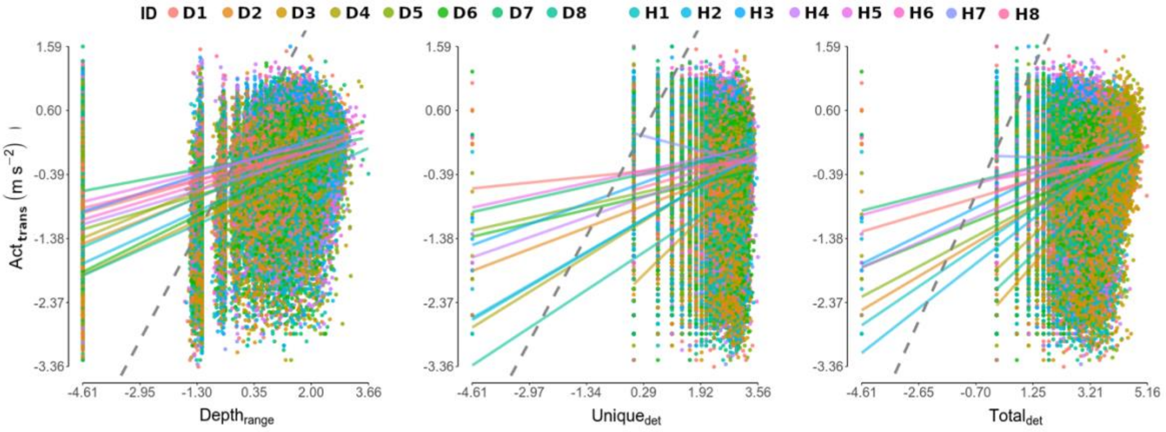

Figure 3.9. Scatterplot showing the relationship between the averaged acoustic activity (Act_{trans}) and the number of unique detections ($Unique_{det}$; A), the number of total detections ($Total_{det}$; B) and the depth-range ($Depth_{range}$; C) at the long-term (~ months). All the variables were calculated at one-hour time bin sizes and log-transformed. In each panel, a solid regression line was added per individual (random factor) to show the fit of the corresponding LME and illustrate possible general trends.

Regarding the effect of ‘Time of day’ on the activity of animals, our results differed between the acceleration biologger and the acceleration transmitter (Table 3.6). In the case of the acceleration biologger, two fish (H7 and H8) showed a trend for higher activity levels during the day while the rest (D8, D5 and H4), were more active during the night (Table 3.6 and Fig. 3.10). In contrast, with the acceleration transmitter data we only found differences in activity between day and night for two of the five fish tested (H4 and D8). Importantly, these two individuals showed the sharpest difference in activity between day and night when observing the effect size for the activity data of the acceleration biologger, ranging between small (H4) and moderate (D8) (Table 3.6) (Tomczak & Tomczak 2014).

Table 3.6. Results of the unpaired two-samples Wilcoxon test (UWT) for differences (‘Two-sided’ test) in activity between the ToD ‘Day’ and ‘Night’ and for a higher diurnal activity (‘One-tailed’) using raw activity data from the acceleration transmitters and activity estimates at SWS of 57 s for the acceleration biologger. $\text{P-val}_{\text{Two-sided}}$ and $\text{P-val}_{\text{One-tailed}}$: P-values for both the “two-sided” and the “one-tailed” UWT, respectively; r: effect size. Significant results (P-value ≤ 0.05) are marked with an asterisk.

	Acceleration biologger			Acceleration transmitter		
ID	**$\text{P-val}_{\text{Two-sided}}$**	**$\text{P-val}_{\text{One-tailed}}$**	**r**	**$\text{P-val}_{\text{Two-sided}}$**	**$\text{P-val}_{\text{One-tailed}}$**	**r**
H4	< 0.001*	1.0000	0.1462	< 0.001*	1.000	0.1836
H7	< 0.001*	< 0.001*	0.660	0.3778	0.1889	0.0196
H8	< 0.001*	< 0.001*	0.1027	0.6399	0.6801	0.0151
D5	< 0.001*	1.0000	0.0612	0.0812	0.9594	0.0611
D8	< 0.001*	1.0000	0.4206	< 0.001*	1.000	0.3902

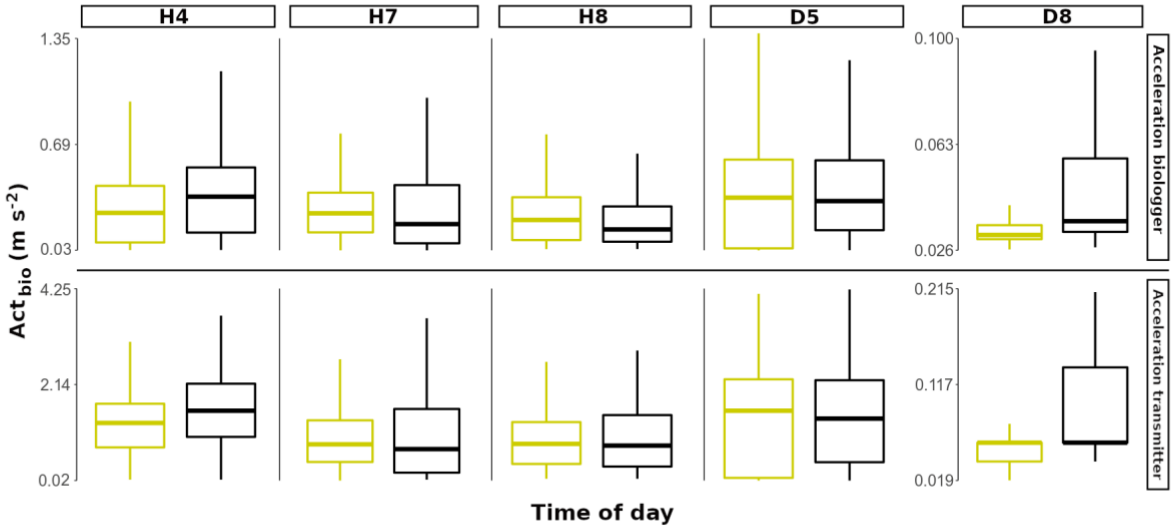

Figure 3.10. Boxplots showing the photoperiod effect (two-level categorical variable: day [yellow] and night [black]) using activity data from the acceleration biologger (upper row) or from the acceleration transmitter (lower row) in the individuals H4, H7, H8, D5 and D8.

4. Discussion

To our knowledge, this is the first study that used two independent tri-axial accelerometers (one internal and one externally anchored) to assess the performance of acoustic telemetry for estimating activity in free-ranging aquatic animals. Our results revealed that acceleration data obtained with accelerations transmitters implanted on *E. marginatus*, a rocky-reef fish species with a high tendency to shelter in caves or crevices, is the only proxy providing an acceptable estimate of activity. The other variables derived from acoustic data that are usually utilized as proxies for activity (namely number of unique and total detections, and depth-range) performed notably worse to infer grouper activity. However, our results also evidenced the need for caution when, for instance, averaging activity into time bins or pointed to possible limitations of this type of data such as lower sensitivity to high activity levels, an individual-dependent sampling bias or lower predictive power associated to that sampling-bias or a lower data-frequency.

The activity estimations made by the acceleration transmitter approached reasonably well those made by the acceleration biologger at sampling-window sizes of 57 s. As noted by Wilson *et al*. (2019), the position and stability of accelerometers play an important role when comparing dynamic body acceleration metrics among individuals. Even when individual differences had a significant effect in the selected model, our results showed that the inclusion of the variability among fish given by the absence of fixed position and stability did not produce a major increase (5%) in the goodness-of-fit. Also, there was a clear non-linear relationship between the acceleration biologger and the acceleration transmitter activity values, which departed from a straight line for activity values measured by the acceleration transmitter lower than 0.058 m s^{-2} in its natural scale. This might be due to the errors introduced during the categorization of continuous RMS values into discrete units before transmission. At very low activity values, the acoustic transmitters conversion can lead to final activity values transformed by up to 100% compared to the original ones (see Appendix D). However, the range of values which suffer this kind of transformation is very small, since it is restricted to extremely low activity values and depends on the tag settings (i.e., the lower the maximum activity measurable by the acoustic transmitter, the higher the activity resolution and less troubling this issue is). Also, it is important to remark that measured activities were consistently higher for the acceleration transmitter than those for the acceleration biologger. This is in line with the significant individual effect in the models and could be evidence of the consequences of using free-ranging (i.e., non-fixed or mobile) accelerometers. The lack of a fixed position and stability of the surgically-implanted accelerometers possibly generates both greater activities and greater differences among individuals in prediction accuracy (Wilson *et al*. 2019). This issue, however, is scarcely considered in the literature (but see Papastamatiou *et al*. 2015), being a common practice to place the acoustic accelerometers internally due to its advantages (Kneebone *et al*. 2018, Brownscombe *et al*. 2019, Hori *et al*. 2019).

The relationship between raw activity data (i.e., non-logarithmized) from the acceleration transmitter and the acceleration biologger also evidenced a loss in accuracy as the activity increased, which could be caused by different constraints. Firstly, recording-frequencies below 10 Hz has been shown to cause drops in the detection probability of certain activities up to 50% (Broell *et al*. 2013). Also, it could be caused by the narrow acceleration range of the acoustic accelerometers so that movements beyond that range could render the same activity estimates as others above it. This issue could be of even greater concern, if it is confirmed that activity is magnified when using free-ranging accelerometers since, beyond it is a common practice in acoustic telemetry studies, the acoustic transmitters have an acceleration range quite restricted. Nevertheless, and similar to what was observed in previous studies (Murchie *et al*. 2011, Kneebone *et al*. 2018, Hori *et al*. 2019), the predominance (otherwise expected, see Priede 1977) of low activity values may have minimised this problem.

Therefore, and even though our analyses highlight the good performance of the activity data of the acoustic transmitters, we also found some potential constraints in the data provided by these devices; notably, derived from the use of free-ranging acoustic accelerometers in very active species. Their use in situations where the goal is to distinguish between extremely low activities should be further investigated to minimize this effect.

The predictive power of activity estimates of the acceleration transmitter remained relatively high regardless of the time bin size considered and ranged grossly between 73% and 80%. These estimates were less accurate at shorter time bins, particularly for low acceleration transmitter activity values. This effect vanished as the time bin size increases and could be related to the fact that the largest the time bin size, the less likely that a low number of detections occurred when the animal was indeed very active for the whole time bin. The higher contribution of the fixed term 'n_{trans}' as the time bin size increased supports this hypothesis, remarking that the linear relationship between activity and number of detections increases as the time bin size enlarges. At the same time, the density functions showed a higher proportion of acceleration transmitter estimates near zero with the smallest time bin (i.e. 6 min) compared to the observed with larger ones (e.g., 120 min). This was to be expected as the fish are less likely to remain immobile for time periods of increasing duration and could evidence that repeated activity measures help to improve the predictive power as the time bin size increased. Also, the number of detections by its own or integrating the interaction term was always included in the selected models, pointing to the relevance of this variable. While at smaller time bins the number of detections was of little relevance, as the size of the bins increased, there was a trend of increasing the risk of making inaccurate predictions of activity with a low number of them. This can be explained because the longer the time bin size, the lower percentage of time the acceleration has been recorded with a low number of detections and the higher the risk of deviating from the real activity measured by the acceleration biologger. Nevertheless, this issue is likely to be of special concern when having high activity estimates with a relatively low number of detections and at large time bin sizes, given the close relationship between a low number of detections and a low activity.

When the use of time intervals is needed for reasons such as time-regularisation (e.g., Taylor *et al*. 2013, Udyawer *et al*. 2017, Runde *et al*. 2020), selecting the bin size should be case-specific. Our results suggest that the selection of an appropriate time bin size appears as a trade-off between choosing a bin small enough to capture the complexities of a dynamic activity pattern, at the expenses of slightly lower accuracy but sufficient duration to get more robust activity estimates, and at the expenses of having a low resolution in the activity patterns described. For large time bins (i.e., >15 min), it would be even possible to establish a threshold for the number of detections, below which activity estimates might be discarded, especially when having high activity estimates with a low number of detections in species like dusky grouper due to their hiding behaviour. Different time bin sizes have been used in the literature: for example, Taylor *et al*. (2013) used 15-min, Runde *et al*. (2020) 30-min, and Udyawer *et al*. (2017) 60-min bins. As we stated above, the selection of a time bin size or of a threshold number of detections could improve the accuracy of the activity estimates, but specific values for the time bin size or the minimum number of detections should be case-specific (goals, species behaviour, tags settings, etc.). Nevertheless, these results could be highly influenced by the behaviour of *E. marginatus* and hence, should be taken with caution.

Our results did not show a consistent sampling bias in all tagged fish. However, in most individuals (four out of six fish) we observed a significant tendency to higher activities for the detection windows (i.e., sampling windows in which there was an activity record from the acoustic transmitter) compared to reference ones (i.e., all the sampling windows). In the remaining two fish, the acoustic activity seemed to accurately represent the activities of *E. marginatus*. Coincidently with the results from goals 1 and 2, here it is also highlighted the importance of the individual differences, as the presence, as well as the magnitude of the sampling bias, varied greatly among them. These results were to be expected given the known hiding behaviour of *E. marginatus*, which may lead to a strong relationship between non-audible periods with low activities and a bias in the activity sampled obtained. These results support recent claims about the individual behaviour as a source of sampling bias (Frair *et al*. 2010, Hebblewhite & Haydon 2010, Caravaggi *et al*. 2020). The lack of a consistent pattern also supports the idea that differences between species and individuals of a given species need to be considered in animal behaviour studies using acoustic telemetry when results are extrapolated to populations (Stuber *et al*. 2013, Caravaggi *et al*. 2020). However, it is worth noting that the effect size was consistently small for all fish (Hattie 2009), which shows that the dusky grouper activity is not sharply different between periods of high and low acoustic signal detection probability because most of the time, they are not likely very active. Hence, we consider that acoustic telemetry provides an adequate representation of the real activity of the population studied within the CPMPA despite the limitations of this technology for providing data when animals are hidden.

Both the short- and long-term models showed that the depth-range, as well as the number of unique or total detections, did not predict or predicted poorly the activity levels in *E. marginatus* when using one-hour time bins. Besides, the visual exploration of the relationship of these activity proxies with the activity measured by the acceleration biologger at other time bin sizes did not suggest that these results could be different using other time bin size. Also, the high relevance of the individual effect for $\text{Depth}_{\text{range}}$, $\text{Unique}_{\text{det}}$ and $\text{Total}_{\text{det}}$ supports this statement, highlighting that its use would be adequate for specific individuals and with lower predictive power. Considering the evidence presented here, the traditional interpretation of acoustic telemetry data to infer activity levels should be taken with caution. The underlying assumption is that a low number of detections relates to an individual hidden and, therefore, less active, which go unnoticed by the receivers (e.g, Arendt *et al*. 2001, Payne *et al*. 2010, Béguer-Pon *et al*. 2015). Another widespread assumption is that changes in depth are indicative of vertical movement and hence closely related to animal activity (Moland *et al*. 2011, Giacalone *et al*. 2015, Villegas-Ríos *et al*. 2017). Although the reasoning behind those scenarios seems logical, it overlooks some common situations. On the one hand, individuals may be outside their shelters (i.e., detectable) but still be stationary. On the other hand, it could be that some fish tend to be very active when closely associated with rocks (i.e., non-detectable) or when they are maintaining their position at a stable depth. All these make, from our point of view, inadvisable to infer activity from acoustic telemetry data if there is no empirical study which supports it, something that has typically occurred in species like the dusky grouper (e.g., Hackradt 2012, Koeck *et al*. 2014, Afonso *et al*. 2016), with the use of accelerometers being the preferred method of measuring activity (Gleiss *et al*. 2010, Wright *et al*. 2014, Wilson *et al*. 2019).

Temporal changes in activity are among the patterns of greater interest in the field of animal behaviour ecology (e.g., Helm *et al*. 2017, Gaynor *et al*. 2018, Coffey *et al*. 2020), so it is necessary to have a deep understanding of the methodologies used for their assessment. Here we found that activity data obtained through acoustic telemetry failed to replicate the results obtained with the acceleration biologger. Using acceleration biologger data we concluded that there were significant differences in activity between day and night for all the individuals, while with the activity data from the acceleration transmitter we could identify differences only in two of them. However, it is important to note that we were able to find differences in activity between day and night using the acceleration transmitter for the two individuals in which the 'Time of day' effect was more evident (i.e., given by the size effect [r] when using data from the acceleration biologger). In the rest of individuals, the differences in activity between day and night were lower, which lead us to believe that data provided by the acceleration transmitter would not lead to invalid conclusions if patterns are evident.

Acoustic telemetry is currently the most feasible tool available to study long-term behavioural patterns of numerous aquatic species (Hussey *et al*. 2015). Furthermore, it allows associating certain bio-telemetry data (e.g., activity or depth) with spatial use, providing an extra value that makes acoustic telemetry one of the most useful tools currently available. However, the interpretation of data obtained with this technology is extremely complex due to the influence of multiple factors, which encompass from the environmental conditions to intrinsic constraints of this technology. We showed the null or poor ability of variables such as the number of detections or the depth range to accurately predict activity, an approach frequently used worldwide with species of different ecology and behaviour (e.g., Arendt *et al*. 2001, Collins *et al*. 2007, Moland *et al*. 2011, Giacalone *et al*. 2015, Skerritt *et al*. 2015, Gandra *et al*. 2018, Christoffersen *et al*. 2019), including *E. marginatus* (Pastor *et al*. 2009, Hackradt 2012, Koeck *et al*. 2014, Afonso *et al*. 2016). Furthermore, our work also corroborated the feasibility of binning activity into time bins larger than the own sampling-windows and suggested that a proper data-handling (e.g., time bin size or setting a minimum number of detections) might improve predictions, but it should be done carefully according to the study design and aims. Nevertheless, we also identified some potential problems with the use of acoustic accelerometers for inferring activity. The absence of stability of the acoustic tags in the coelomic cavity, their low acceleration range and recording-frequency, or even the own behaviour of tagged individuals are aspects to consider when making a proper interpretation of activity data provided by acoustic accelerometers, as they can affect their reliability. Given the existing literature about the performance of acceleration data as an activity proxy (e.g., Gleiss *et al*. 2010, Wilson *et al*. 2019) and our own results, we consider that, nowadays, the only available method suitable to estimate aquatic animal activity in acoustic telemetry is through the use of built-in accelerometers. Alternative methods that are not based on the use of acceleration data should be tested empirically. A good interpretation of acoustic telemetry data is crucial from a conservation perspective (Brownscombe *et al*. 2019), so we claim for additional efforts in the line of this study which help to fully understand the performance of the acoustic telemetry as a method to infer animal activity.

Chapter IV

Environmental drivers and SCUBA-diving presence effects on the activity of a marine top predator fish using biologging and Hidden Markov Models

Environmental drivers and SCUBA-diving presence effects on the activity of a marine top predator fish using biologging and Hidden Markov Models

ABSTRACT

Activity patterns is one of the most important but less studied behavioural traits of aquatic animals, especially regarding the likely influence of human activity on this feature. In this study, we simultaneously evaluated the effect of a series of environmental variables (photoperiod, time of day, lunar phase, temperature, storms, intensity and direction of marine currents), and of the presence of SCUBA divers on the activity patterns of the dusky grouper (*Epinephelus marginatus*) in a Western Mediterranean marine protected area. For this purpose, we used acceleration data obtained from acoustic telemetry in both a no-take zone (where diving is not allowed) and a control zone (highly frequented by divers) in a total of 16 grouper individuals (8 in each zone). Data were analysed by Hidden Markov Models, which allow to estimate the probability distribution of resting *vs*. active (hidden) states, as well as the transition probabilities between both discrete behavioural states. The environmental variables for which dusky grouper showed a clear and relatively consistent response among individuals were storms (which increased their activity), time of day (being more active at night than during the day), temperature and current intensity (whose increases triggered increases in activity), and lunar phase (with tendencies to reduce activity as the full moon approaches). Nevertheless, some variability was observed among individuals, which was relatively high for the other environmental variables whose influence was explored, stressing the importance of fish personality. Similarly, the presence of divers caused different activity patterns among individuals (so that it could be more or less active, or remain neutral), so there is no clear response to this anthropogenic factor. Surprisingly, the time of day when divers were in the water was associated with higher activity in some individuals tagged in the no-take zone, located 1.2 km from the dive site, indicating that other drivers related to diving activity (such as noise from boat engines) could be affecting grouper behaviour. Remarkably, some of the results found differ from the widely accepted assumptions for dusky grouper concerning their activity patterns, evidencing the importance of advances in biologging and the need for new studies using enhanced methodologies.

1. Introduction

Human activity is strongly affecting marine ecosystems in a myriad of ways (e.g., Halpern *et al*. 2008, 2019). Human-induced change in animal behaviour is one of which raises major concern since how animals interact with their environment determines numerous ecological functions and ultimately alters community structure and ecosystem resilience (Tuomainen & Candolin 2011, Sih *et al*. 2012, Wilson *et al*. 2020). Animal behavior has been documented to be a key aspect to consider when, among others, designing wildlife management measures (e.g., Greggor *et al*. 2016, Alós *et al*. 2019, Dwyer *et al*. 2019), evaluating the viability of commercial activities (Terrasson *et al*. 2016, Føre *et al*. 2017, Muñoz *et al*. 2020), and understanding the evolutionary implications of individual variation of behaviour (Sih *et al*. 2011). Understanding how the footprint of human affects animal behaviour is therefore pivotal for conservation as well as for defining sustainable management strategies (Arlinghaus *et al*. 2017).

Given the tremendous expansion of nature-based tourism worldwide (Knight 2009, Prideaux & Cooper 2009, Balmford *et al*. 2015), the study of the behavioural changes induced by recreational activities has been a subject of particular interest (e.g., Kovacs & Innes 1990, Geffroy *et al*. 2015, Bateman & Fleming 2017). In aquatic ecosystems, a highly relevant activity is recreational diving, as it usually involves close interactions between humans and wild animals. This type of activities entails the risk of habituation of animals to human presence (e.g., Valerio *et al*. 2019), as they do not perceive any hazard, thus relaxing their fleeing mechanisms and making them more vulnerable to speargun fishers and poachers (Januchowski-Hartley *et al*. 2013). In an opposite way, the human presence might cause variations in some of the animal behavioural traits such as their activity or exploration patterns (Alós *et al*. 2012, Titus *et al*. 2015, Martorell-Barceló *et al*. 2018). Given the potential ecological impacts of any diver-induced persistent variation on behavioral traits (Arlinghaus *et al*. 2017), it is crucial to further assess this issue by describing its magnitude and consequences (Wilson *et al*. 2020).

A major technological challenge addressing human-induced behavioural changes in aquatic systems is the acquisition of behavioural data on free-living aquatic animals (Hussey *et al*. 2015). In recent years, aquatic animal tracking has enabled scientists to raise more complex questions about human-induced effects on aquatic animals' behaviour, by providing the tools to accurately track free-living fish in both freshwater and marine systems (Madrak *et al*. 2016, Bradley *et al*. 2017a, Mourier *et al*. 2020; Hussey *et al*. 2015). Acoustic telemetry technology allows tracking individuals over relative long periods of time and to obtain data that are impossible to collect using more traditional techniques, which makes this method a tremendously valuable source of data in marine fish (Brown *et al*. 2013, Donaldson *et al*. 2014, Hussey *et al*. 2015). However, to the best of our knowledge, the only study to date that explicitly assesses the effect of SCUBA divers on fish behaviour based on long-term individual tracking is Bradley *et al*. (2017b), which detected no effect of diving on the spatial distribution of the blacktip reef shark *Carcharhinus melanopterus* in Palmyra Atoll (central Pacific). This study highlights the need for more research on this issue, studying other taxonomic groups and behavioural traits (Conrad *et al*. 2011) and combining the simultaneous tracking of wild fish with the monitoring of the human presence.

Variation in animal activity patterns might be one of the most important behavioural traits affected by recreational diving, as this could alter species interaction, having, therefore, implications for individual species survival, and lastly, causing cascading ecological consequences when the target species is a top predator (Hertel *et al*. 2017, van der Vinne *et al*. 2019, Wilson *et al*. 2020). A number of studies have shown how human activity can provoke shifts in animal activity patterns (e.g., Berger 2011, Alós *et al*. 2012, Leblond *et al*. 2013, Benítez-López 2018, Gaynor *et al*. 2018, Ladle *et al*. 2018). Most of them, however, focus on examining human-induced changes in spatial patterns of activity (i.e., measuring changes in their home ranges, territories, or migration patterns), but not in temporal ones. Furthermore, the term "activity" has led to a wide range of descriptions of disturbance-induced effects on activity in the scientific literature, making it difficult to interpret and draw general conclusions. For instance, Ladle *et al*. (2018) used independent counts from camera-traps in the bear species *Ursus americanus* and *Ursus arctos*, and Christoffersen *et al*. (2019) the number of detections in the round goby *Neogobius melanostomus*, both, to assess their daily activity patterns. In telemetry, a variety of data-types (travelled distances, number of detections, depth-range, acceleration, etc.) can be used to define “behaviours” or “activities” (e.g., “foraging”, “transit”, “resting”, “hidden”), and therefore, changes in any of these traits can be catalogued as a change in activity patterns (e.g., Dean *et al*. 2013, van Beest *et al*. 2019, Itakura *et al*. 2017, Coffey *et al*. 2020). However, from a thermodynamic point of view, one could ask whether all these data-types or behaviours define activity correctly, since in many cases the patterns found at the behavioural level may not correlate with the energy consumption patterns of the animals (Campos-Candela *et al*. 2019, see also Chapter 3 of this thesis). This is intimately linked to the current temporal and spatial resolution constraints of technology used in animal behaviour research, which could derive in situations such as individuals spending more energy when they cover smaller areas than when they cover larger ones. In this regard, accelerometer technology could solve the problem of generating activity-related data to estimate human-induced changes, given the proved closed relationship between acceleration of animals and their energy consumption (Qasem *et al*. 2012, Wilson *et al*. 2019).

In addition to the challenge of generating behavioural data in free-living animals, another problem exists on distinguishing human-induced effects from those caused by environmental stimuli (Levitis *et al*. 2009), which can interact and lead to confusion (Kaczensky *et al*. 2006, Theuerkauf *et al*. 2007). Therefore, there is a need for simultaneously monitoring both environmental and anthropogenic factors when assessing the human influence on animal behaviour (Ordiz *et al*. 2014, Garant 2020). A statistically powerful experimental design typically used when studying human-induced animal behaviour change is the so-called Before-After-Control-Impact (BACI) design (Green 1979, Underwood 1991, Smokorowski & Randall 2017). Though, it is usual to use either temporal (Before-After [BA] designs) (e.g., Watson & Harvey 2007, Barker *et al*. 2011) or spatial (Control-Impact [CI] designs) (e.g., Titus *et al*. 2015, Mourier *et al*. 2020) controls, assuming that the factors not subject to analysis remain constant between levels to be compared, and therefore, being the factor under study (e.g., human-presence or provisioning) the cause of the possible differences (Smokorowski & Randall 2017). While temporal BA designs allow the assessment of behavioural changes against the presence of human activity (i.e., differences between before and after the activity), CI designs enable to confirm that those changes are not due to other causes (i.e., only present in the impacted area).

Here we aimed to analyse the environmental and SCUBA-diving induced effects on the activity patterns of the dusky grouper (*Epinephelus marginatus*, Lowe 1834), a top predator fish species of high ecological and socio-economic interest. Specifically, by using Hidden Markov Models (HMM), we evaluated the possible effects of both different environmental variables and the presence of SCUBA-divers using high temporal resolution activity data and a CI design. Our main hypothesis is that the effect of SCUBA-divers on the activity patterns of dusky groupers is likely marginal or null, given that the study was performed in a marine fisheries reserve where recreational diving has been intensely promoted since 1995 and spearfishing is not allowed. Besides, our target-species may easily lose its fear due to its low predation risk, especially from medium sizes, which increases the odds to its habituation to the SCUBA-diving activity. Likewise, this study arouses additional interest because of two main reasons. Firstly, it was conducted in an area where recreational diving and artisanal fishing coexist, and any behavioural change could trigger adverse consequences for the latter one, an activity whose conservation should be a priority given its high cultural value and low environmental impact (García-de-la-Fuente *et al*. 2016). Secondly, there is not yet clear evidence on the environmental drivers of dusky groupers activity patterns, which is mainly due to both the technical limitations of the previous studies (e.g., no acceleration data) and the complexity of the topic under study (Pastor *et al*. 2009, Hackradt 2012, Koeck *et al*. 2014, Afonso *et al*. 2016).

2. Materials & Methods

2.1 Study area and species

The present work was carried out on a top predator fish, the dusky grouper *Epinephelus marginatus*, at the Cabo de Palos – Islas Hormigas Marine Fishery Reserve (CPIH-MFR) (Fig. 4.1). This MPA was created in 1995 and is located off the coast of Cabo de Palos (Murcia, Southeast Spain), in SW Mediterranean Sea.

Rectangular in shape, it is 7.3 km long, 2.3 km wide and has an area of 1898 ha. It harbours a series of seamounts lined up in the west-east direction, two of which reach the surface and result in the Hormiga and Hormigón islets (Fig. 4.1). The CPIH-MFR has two protection levels: a fully protected or no-take zone (NTZ) area of 270 ha, defined by a circle of 500 m radius from the island "Hormiga", and a partially protected area, (PPA) that encompass the remaining surface. The NTZ is in the core of the MPA and no activity, except scientific research, is allowed. In the PPA only artisanal fishing and SCUBA-diving activities are authorised. SCUBA-diving activity is organised in turns of one hour, and it is only allowed during the daylight. The schedule and the maximum number of divers per seamount change between the high (summer) and the low (winter) season, with a daily maximum number of SCUBA-divers of 70 in summer. Although fishers and divers can be present simultaneously in any of the PPA seamounts, fishers usually avoid operating when diver frequentation is high, both for safety reasons and because they believe that the divers negatively impact their catches. The study was developed in two zones under different protection levels: the seamount "Bajo de dentro" (BD), where diving is allowed, and the "Hormigón" islet (HG), where diving is forbidden. Both zones are contiguous (~1.2 km away from each other) and they are similar in terms of size and bathymetry.

The dusky grouper is a demersal species strongly associated with rocky substrates, where it usually seeks small cavities for shelter (Barreiros & Santos 1998, Harmelin & Harmelin-Vivien 1999). In this species, either the depth-range distribution, mobility degree and feeding habits vary according to age (Condini *et al*. 2018): younger individuals tend to live at shallower depths, be more mobile and base their diet on small crustaceans (Azevedo *et al*. 1995, Barreiros & Santos 1998, Harmelin & Harmelin-Vivien 1999); as the individuals grow, their depth range becomes much wider (up to 250 m deep) and their diet diversifies, but they also become more sedentary and territorial (Condini *et al*. 2018). Dusky groupers can be found on both the eastern and western Atlantic Ocean, the eastern part of the Indian Ocean, and throughout the Mediterranean Sea (Heemstra & Randall 1993, Condini *et al*. 2018). Its presence is often considered a symptom of good ecological conditions since its behaviour and feeding habits modulate the ecosystems in which it is present (Condini *et al*. 2018). This, together with the fact that it is a highly valued species by humans (both for watching and consumption), with a high site-fidelity, long life-span (up to 60 years), slow growth (Manooch 1987, Reñones *et al*. 2007, Condini *et al*. 2014) and late sexual maturity (Reñones *et al*. 2010), has resulted in its inclusion since 2004 as "Vulnerable" species on the Red List of threatened species of the International Union for the Conservation of Nature (IUCN). In many MPAs, especially in the Mediterranean Sea, groupers-watching is a phenomenon of special interest for SCUBA divers. Therefore, the confirmation of the recovery of this species within marine protected areas (Hackradt *et al*. 2014, Giakoumi *et al*. 2017) is of great interest in conservation as it helps to create a positive perception of these spaces in society.

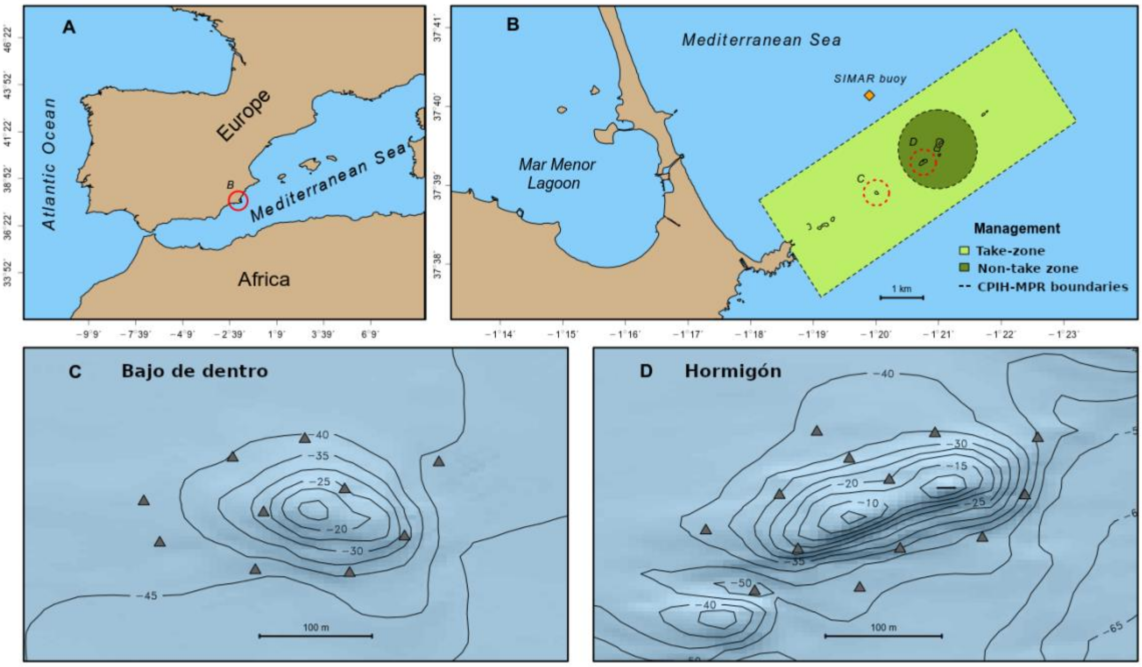

Figure 4.1. Maps illustrating the general location of the study area (i.e., the Cabo de Palos - Islas Hormigas Marine Fishery Reserve, SE Spain) (A), the location of the study sites (Bajo de dentro [BD] and Hormigón [HG]) and the SIMAR buoy in the context of that MPA (B), and the bathymetry as well as the acoustic receivers' arrays (as triangles) both in BD (C) and HG (D). Figure B also shows the spatial distribution of the two levels of protection of the MPA.

2.2 Fish handling and tagging

A total of 16 dusky grouper individuals (8 in HG and 8 in BD) were caught using artisanal baited traps made of wicker, a technique that avoids the damage and stress generated using line and hook. They were all adults ranging in size between 50 and 92 cm (total length) and an estimated weight between two and 12 kg (Reñones *et al*. 2007, 2010). Once fish were on board, they were placed in a tank with seawater regularly aerated and replaced. The swim bladder was deflated with a hollow needle prior to handling. Once stabilized, fish were moved to other tank containing an anaesthetic solution (40 $mg{\cdot}l^{-1}$; 1-part clove oil emulsified in 9-parts ethanol). The anaesthesia procedure followed ethical recommendations by Neiffer & Stamper (2009). Fish were maintained in the anaesthetic solution until loss of balance and lack of response when pressing the caudal peduncle was observed. Once anaesthetized, they were placed on an ichthyometer for the surgery. The head was covered with a wet towel and cold seawater was run through the gills during the surgery. First, we made a 3-cm incision in the ventral cavity using a sterile surgical blade, where we introduced a V13AP acoustic transmitter (Vemco Inc). After implantation, we sealed the surgical incision using surgical glue to prevent its permeability. The transmitters had a length of 4.8 cm and a weight in air of 13 g, and therefore, never exceeded 1% of the body mass of individuals. Povidone-iodine and an antibiotic spray were spread on the manipulated area. The transmitters were always placed keeping the same orientation in all individuals. After a short recovery, fish were released in the same area where they had been captured. Fish behaviour was checked by a diver for at least 10 minutes, from their released at the surface to the moment in which they leaned on the bottom or they hid in some cave. Handling and tagging procedures followed recommendations described by Wagner *et al*. (2011) and were performed by authorized personnel. We also had the pertinent permits both, for working in CPIH-MFR and for ethical aspects related to animal welfare.

2.3 Tags settings and passive monitoring

The V13AP is a coded acoustic transmitter bearing a built-in tri-axial accelerometer and pressure (depth) sensor (model V13AP, Vemco, Inc.). They were set to send activity and depth records alternatively, with an average random delay among consecutive emissions of 120 s and within a fixed range of 60 s - 180 s. The V13AP tag uses as activity proxy the root mean square (RMS, Eq.1), which averages the tri-axial acceleration over a customised sampling window (Cooke *et al*. 2016):

$$RMS = \frac{\sqrt{\sum_{i=1}^{n}\left(X_{dyn}^2 + Y_{dyn}^2 + Z_{dyn}^2\right)}}{SWS * \omega} (Eq. 1)$$

where X_{dyn}, Y_{dyn} and Z_{dyn} represent the dynamic acceleration (m s^{-2}) in the X, Y and Z axes, respectively; *SWS* represent the size of the sampling-window (in seconds) and ω, the recording frequency (Hz). In our case, the coded transmitters were set to record acceleration over sampling-windows of 57s, with the RMS values ranging between 0.0 m s^{-2} and 4.91 m s^{-2}, and with a resolution of 0.019 m s^{-2}. The acceleration range of the V13AP accelerometers is ± 29.4 m s^{-2}. The pressure sensor from the V13AP was set to the "full-scale" pressure option of 68 m, with an accuracy and resolution of 3.4 m and 0.3 m, respectively. These acoustic transmitters have an output power of 153 dB and an expected battery life of 214 days. To account for differences in clock drift among receivers, they were time-synchronised using VUE software of Vemco Ltd.

To quantify the long-term activity patterns of dusky groupers, two arrays of acoustic receivers (model VR2W, Vemco, Inc.) were deployed, one in HG (n=13) and the other in BD (n=10). Receivers were placed in both areas at depths between 20 and 50 m. Besides, they were deployed less than 100 meters apart between each other, according to the recommendations for reef areas made by Welsh *et al*. (2012) and considerably below the estimated detection range (~250 m) in the area by Hackradt (2012). This design ensured to both cover completely the suitable habitats for dusky grouper in those areas as well as increase the reception probability of the acoustic signals despite the acoustic complexity of the area (Fig. 4.1). Each receiver was fixed to a rope attached to a buoy floating in mid-water, so that it was suspended about 5 m above the bottom, the rope being tied to an iron bar which in turn protruded from a concrete block (Fig. 4.2). This design made it possible to reduce the impact of these structures on the fishing activity taking place in the area, to reduce the environmental noise around the receivers associated with surface waters, and to allow better acoustic conditions for the receivers compared to their placement over the bottom directly. For each acoustic detection, the receivers registered date and time, a unique ID for each fish, as well as either an acceleration or a pressure value.

The VR2W receivers were removed at the end of the study period, and the data collected were downloaded with the software V.U.E. (Vemco Inc). Isolated detections occurring within a 24-h window where considered spurious and therefore removed from the dataset. Because the receivers were very close to each other, the multiple detections (< 60 s) from a single ID were filtered leaving only the first detection. Lastly, the activity data were averaged at regular bins of 15-minutes before the analysis.

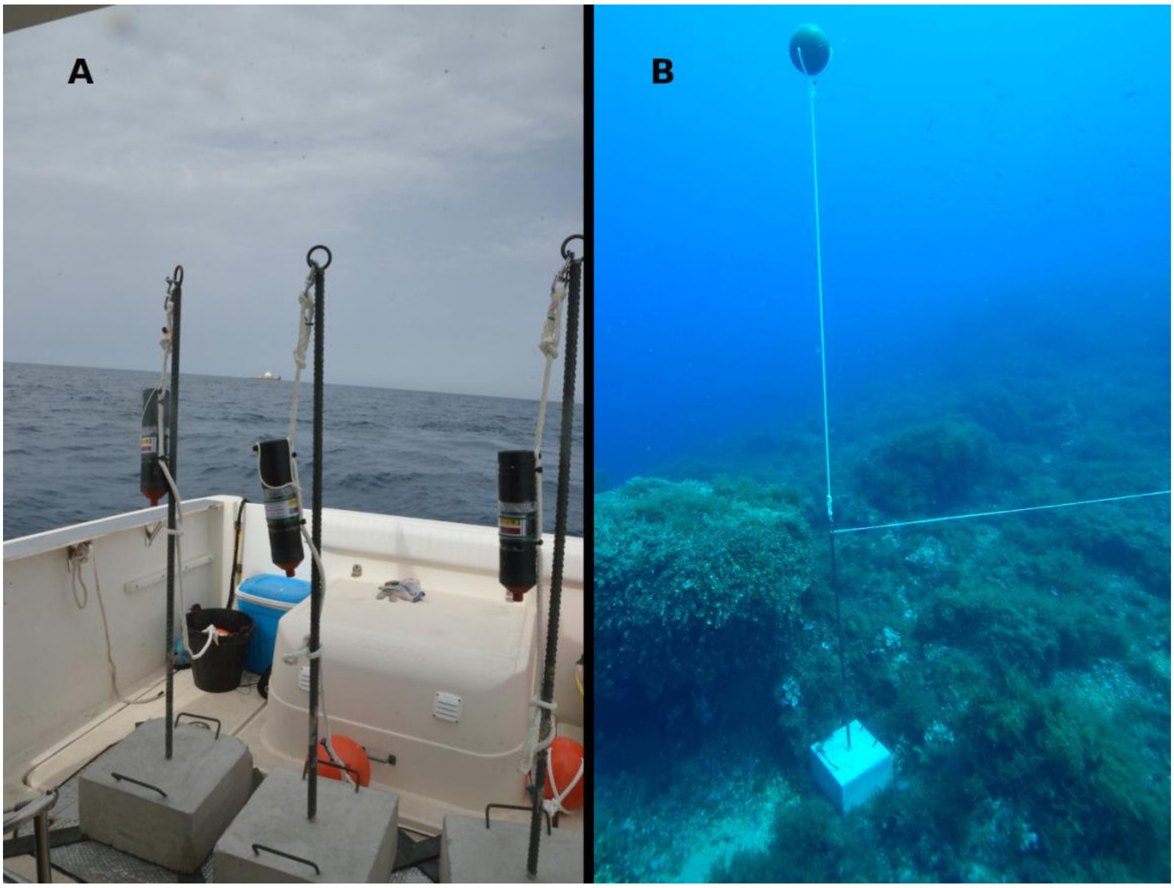

Figure 4.2. Structures in which the VR2W receivers were deployed. The base is a concrete structure weighing approximately 60 kg, under which metal supports were placed to prevent the structures from tipping over. From the concrete base, a 1.3 m iron bar raised over which a 4 m long rope was placed on which the VR2W and a buoy were fixed. The buoy was placed at its upper end to ensure that the line was kept upright. The left (A) and right (B) sides show the moorings' appearance before and after their deployment, respectively.

2.4 Presence of SCUBA divers and environmental data collection and processing

The variable ‘presence of SCUBA divers’ was based on hourly data on the number of divers in the BD (i.e., the ‘Non-take’ zone where SCUBA-diving is allowed), from which we created a 15-min time-bin variable indicating the presence of divers (‘No’/’Yes’) at that moment. Data were provided by the Regional Ministry for Water, Agriculture, Livestock, Fisheries and Environment (Autonomous Government of the Region of Murcia), which is the competent management body of the CPIH-MFR.

Regarding the environmental variables, we selected the following: time of day, water temperature, marine current intensity and direction, storms, moon and photoperiod, selected from a set of more than 20 available variables through a principal component analysis (PCA), which allowed us to identify those which captured most of the environmental variability. Lastly, we used the variance inflation factor (VIF) analysis to assess that none of the selected variables was highly correlated (VIF<3) (Zuur *et al*. 2009). The PCA and VIF analysis were performed using the ‘prcomp’ and ‘vif’ functions included in R-base and the R-package Car (v3.0-10), respectively. The 'time-of-day' was categorised into two levels: 'Day' and 'Night'. To do so, we used the local sunrise and sunset times using the 'getSunlightTimes' function from the R-package suncalc (Agafonkin & Thieurmel 2017). Since each fish had a different depth profile (Fig. 4.3), the water temperature value associated to each activity record corresponded to the hourly mean water temperature at the fish depth based on four temperature sensors (model HOBO Water Temp Pro v2, Onset Inc) placed at 5, 15, 25 and 35 m depth in HG. The marine currents data were provided by the Western Mediterranean OPerational forecasting system (WMOP; Juza *et al*. 2016), developed at the Balearic Islands Coastal Observing and Forecasting System (Tintoré *et al*. 2013), which is based on a regional ocean configuration of the ROMS model (Shchepetkin & McWilliams 2005) and has a horizontal and vertical resolution of 2 km and 2 meters, respectively, as well as a temporal resolution of 3 hours. The maximum depth assumed by the models in the study area was of 41 m. After exploring the individual depth profile (Fig. 4.3), we averaged both the intensity and direction of the marine currents between 13 and 41 meters depth. The direction of marine current was categorised in two levels according to the two main current directions in the area (Gutiérrez *et al*. 2009): 'North' and 'South'. The storm conditions were described by wave height (m) data, provided by the Spanish public organism ‘Puertos del Estado’ within the data framework SIMAR. This organism, in coordination with the Agencia Estatal de Meteorología (AEMET), uses a wave generation model (WAM, Günther *et al*. 1992) along with real data from a Spanish coastal buoys network to interpolate a temporal series of swell data for a set of coordinates (WANA points grid). Here, we used data from a WANA point located 2 km apart (SIMAR code: 2076092, Fig. 4.1). The moon effect was determined through its luminosity using the 'getMoonIllumination' function from the R-package suncalc (Agafonkin & Thieurmel 2017). This function enables to calculate the fraction of the moon that is illuminated given a date, and the values range between 0 (new moon) and 1 (full moon). Photoperiod was calculated daily as the proportion of light time concerning 24 hours a day (i.e., a value of 0.6 indicates that 60% of a day there was sunlight) using the 'getSunlightTimes' function of the R 'suncalc' package.

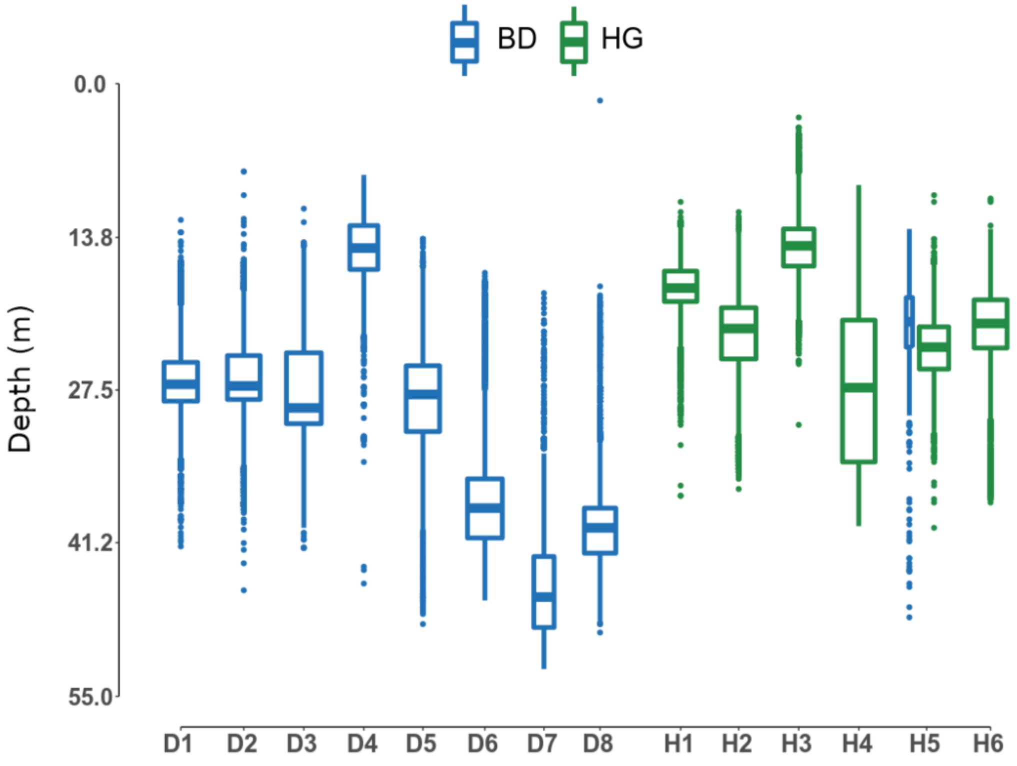

Figure 4.3. Boxplots of the depth (averaged at 15-min time bins) of the individuals of *E.marginatus* under study. Different colours and box-sizes were used to indicate both the study area where they were detected and the sample size.

2.5 Data analysis

We used a Hidden Markov Model (HMM) to test whether the activity of *E. marginatus* was affected by either some of the environmental variables described above or by the presence of SCUBA-divers. A separate analysis was conducted with each individual to unmask possible cross patterns among them. HMMs assume the existence of an unobserved (hidden) sequence of states (finite-state Markov chain of first order) that can be derived from an observed one (Baum & Petrie 1966) and has been previously applied to Acoustic Tracking data in marine systems to detect behavioural states and the effect of different types of disturbances (Alós *et al*. 2017, Coffey *et al*. 2020, Knaap *et al*. 2021). In our case, we used the average activity at regular time-steps (15-min) as metric of fish behaviour, being 'φ_t' the mean activity for a given time t. The sequence of φ_t (Markovian chain) was used as input in the HMMs that, automatically, create a sequence of two behavioural states: active ('A') and resting ('R'), as the histograms of the data suggested (see Fig. 4.4). Therefore, the latent Markov process is used to model these discrete behavioural states and allows to estimate the parameters for both the state-dependent probability distributions (i.e., the distribution parameters per each state) and the transition probabilities between states (A→A, A→R, R→R, R→A).

The state-dependent distribution parameters, the regression coefficients for both the state-dependent distributions and the transition probabilities (β_0 and β_1), and the initial probability distribution (Δ) were estimated using the function 'fitHMM' from the R-package 'momentuHMM' (McClintock & Michelot 2018), which applies a numerical maximization of the log-likelihood (Zucchini *et al*. 2016). We selected a gamma distribution with parameters μ (mean) and σ (standard-deviation) to describe φ_t after exploring its distribution through histograms. We examined different sets of initial values for the HMM parameters, observing that all of them meet in similar final parameters estimation, which suggests that the HMM found the global optimum of the likelihood function. After that, we made histograms and qq-plots of the pseudo-residuals of the HMMs to check both normality and the goodness-of-fit of the HMMs.

Particularly, we assessed the effect of both the environmental variables and SCUBA-divers presence through a two-step process. First, we compared the performance of different HMMs fitted to the activity data of *E. marginatus* that included different combinations of the variables of interest using the scores of the Akaike Information Criterion (AIC), selecting the model with the smaller AIC as the one with better performance. Secondly, we explored the regression coefficients estimates (± CI) for both the state-dependent distribution probabilities and the transition probabilities, which inform us about the type and magnitude of the effects whenever they were present (the zero was not included within the CI). The regression coefficients estimates and their CI were obtained using the function ‘CIbeta’ from the R-package ‘momentuHMM’ (McClintock & Michelot 2018). In the case of the environmental variables, we explored the effect of the ‘time-of-day’, ‘water temperature’, ‘current intensity’, ‘current direction’, ‘storms’, ‘moon’ and ‘photoperiod’ on the activity patterns of dusky groupers, and model selection was performed comparing HMMs which used distinct combinations of these variables with a null-HMM (i.e., no variables). In the case of the effect of the presence of SCUBA-divers, we selected only data for the time of day ‘Day’ since the SCUBA-diving activity is not allowed in the study area at night, and we tested it both in the area where SCUBA-diving is allowed (BD) as well as in which it is not (HG), thus serving as control individuals, hypothesising that this effect will be only present in individuals from BD. Here, the model selection was performed comparing two fitted HMMs, with and without the presence of SCUBA-divers, but both including all the environmental variables that had a significant impact on dusky groupers’ activity (except ‘time-of-day’).

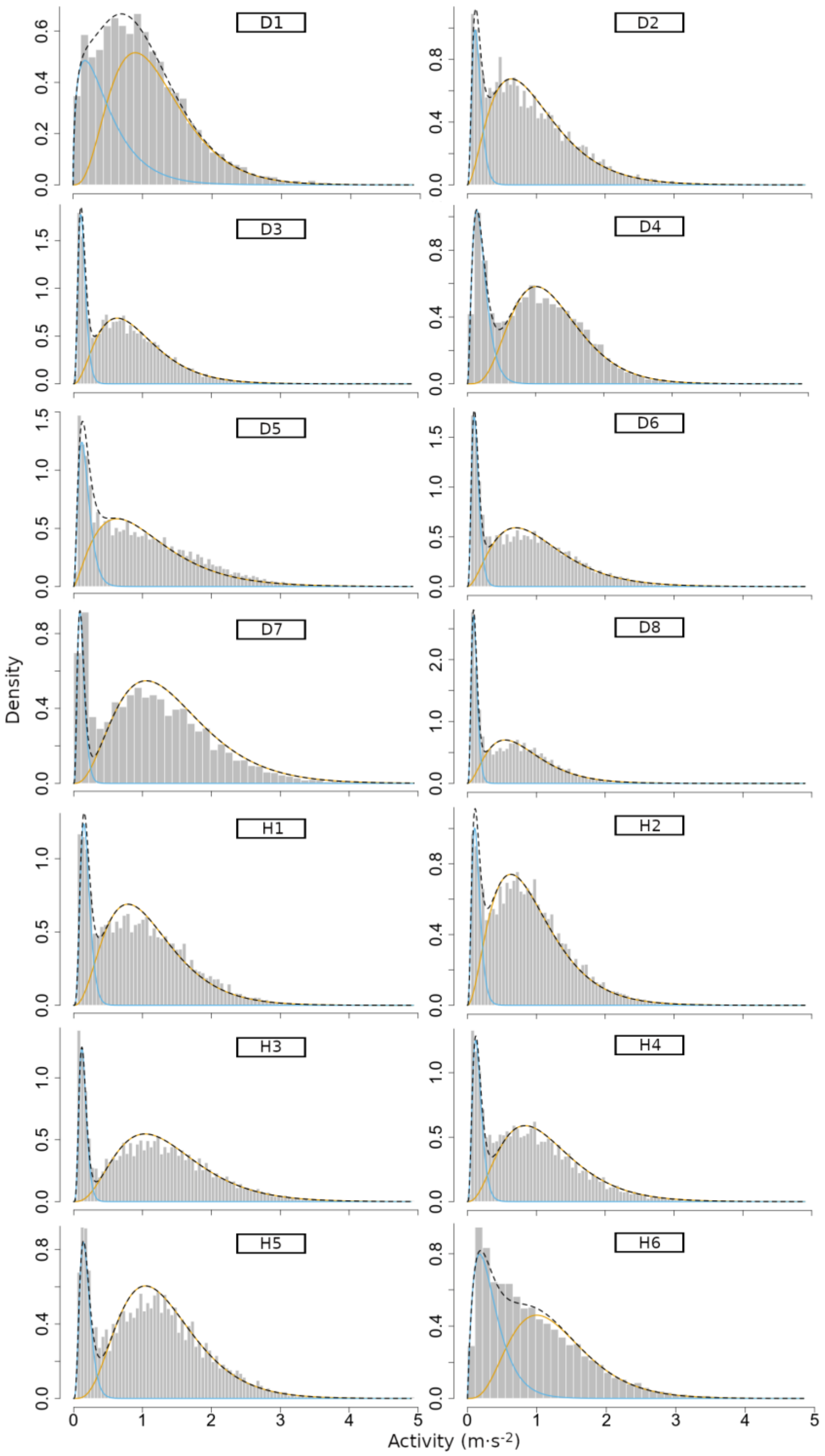

Figure 4.4. Histograms of the activity (m s^{-2}) data (averaged at 15-min time bins) along with the estimated state-dependent probability distributions for the individuals of *E.marginatus* under study for specific environmental conditions (Temperature = 17.78 degrees celsius, Current intensity = 0.1 m s^{-2}, Current direction = North, Storm [~wave height] = 0.5 m, Moon = 0.49, Photoperiodism = 0.49 and Time of day= Night). Different line colours were used to show the probability distribution of the states 'Active' (orange) and 'Resting' (blue). Furthermore, a dashed black line was added to show the overall probability distribution.

3. Results

We obtained 1,030,688 unique activity detections (i.e., without considering receptions by multiple receivers of a single acoustic transmission) from the V13AP tags implanted in the 16 dusky groupers studied. We excluded two individuals from HG (H7 and H8) from the analysis since we only obtained data for the first month after their release. To ensure homogeneity in environmental conditions among individuals, we selected data between the 2nd September 2016 and 16th March 2017, obtaining finally 198,823 15-min time bins with activity information distributed heterogeneously among individuals (Fig. 4.5, Table 4.1). Actvity data were approximately bi-modal for all individuals, coinciding with our assumption of the two-state activity states (resting and active), and rarely reached the maximum activity value (i.e., 4.9 m s^{-2}) configured for the V13AP (Fig. 4.4). For the active state, the initial values for the mean (μ) and variance (σ) parameters in the HMMs were set both to 1.2, while they were both set to 0.13 in the case of the resting state. The fitted μ and σ for both states showed to be independent of the initial values of the parameters. Concerning the variable presence of SCUBA-divers, data were distributed heterogeneously throughout time and among hours, with divers being concentrated mostly in late summer and during the weekends in the CPIH-MFR (Fig. 4.6 and Fig. 4.7).

Table 4.1. Summay of the tracking data results obtained from the 14 dusky groupers considered in our study. For each individual (ID), we indicate the study area (Site) in which it was tagged (and released), its total length (TL) in cm, the number of 15-min time data bins (n) in which we obtained activity data for the study period, the percentage (%) of 15-min time bins for which we have activity data considering for each ID as the initial and the final time the first and the last time they were detected, and the range of average activity (m s^{-2}) values at 15-min time bins (Act_{range}).

ID	Site	TL	n	%	Act_{range}
D1	BD	78.0	15848	84,4	0.029 – 4.901
D2	BD	92.0	15061	80,2	0.038 – 4.901
D3	BD	84.5	16997	90,5	0.038 – 4.901
D4	BD	89.0	11029	89,3	0.038 – 4.901
D5	BD	74.0	15662	83,4	0.038 – 4.574
D6	BD	72.5	17032	90,7	0.029 – 4.901
D7	BD	71.0	5827	50,6	0.038 – 4.901
D8	BD	82.5	13877	73,9	0.029 – 4.901
H1	HG	75.0	14858	83,4	0.038 – 4.536
H2	HG	85.0	16687	88,9	0.038 – 4.901
H3	HG	50.0	13117	69,9	0.038 – 4.901
H4	HG	63.0	14990	84,2	0.038 – 4.901
H5	HG	64.0	12693	73,9	0.038 – 4.901
H6	HG	54.0	15145	81,5	0.038 – 4.843

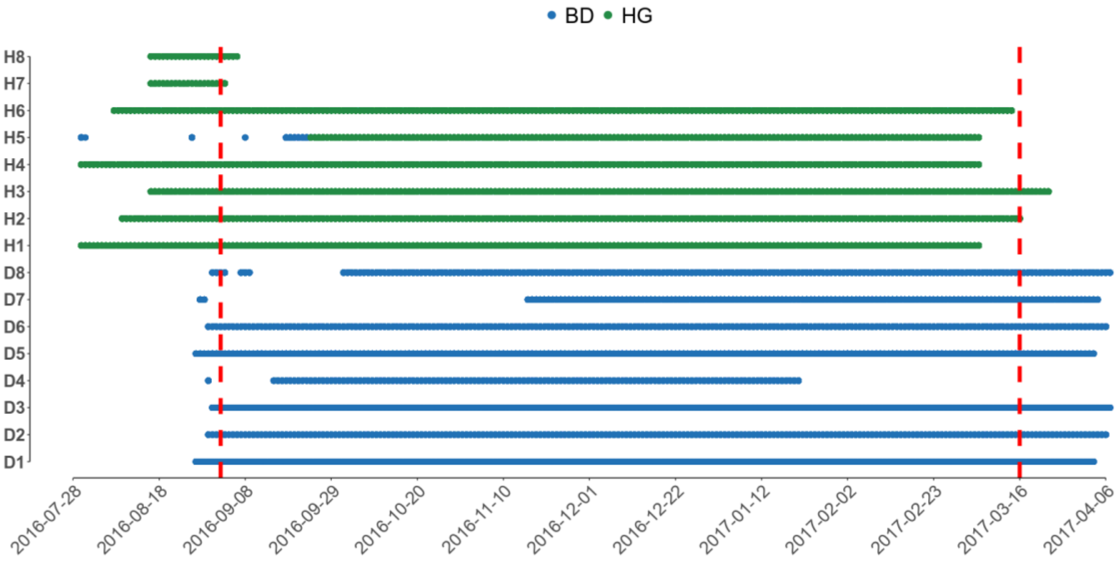

Figure 4.5. Plot showing the residency time periods for which we obtained data from the V13AP acoustic transmitters implanted in *E. marginatus*. The letter that encodes each individual indicates the place where the individuals were marked and released (D: BD in blue, H: HG in green). The lines are coloured to identify where they were detected (blue: BD, green: HG). The vertical red dashed lines indicate the initial and final threshold time selected to ensure homogeneity in environmental conditions among individuals.

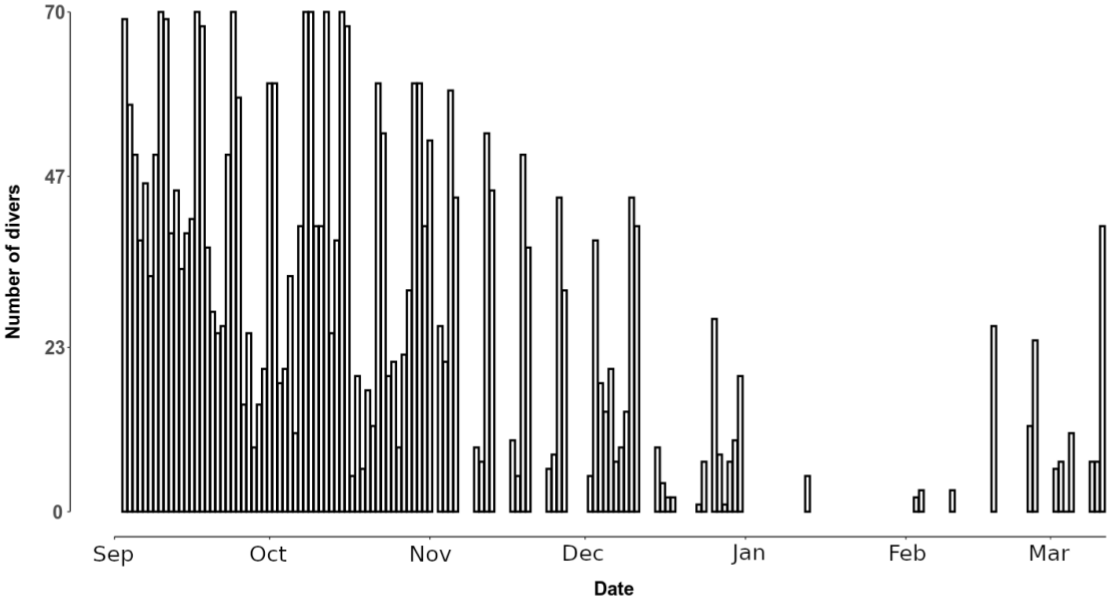

Figure 4.6. Barplot indicating the daily number of divers in the study area where scuba-diving was allowed (i.e., the BD) throughout the study period (from 2nd September 2016 to the 16th March 2017). Data was provided by the local government to whom the scuba-diving centers inform about the time slot and the number of divers that will visit the dive site.

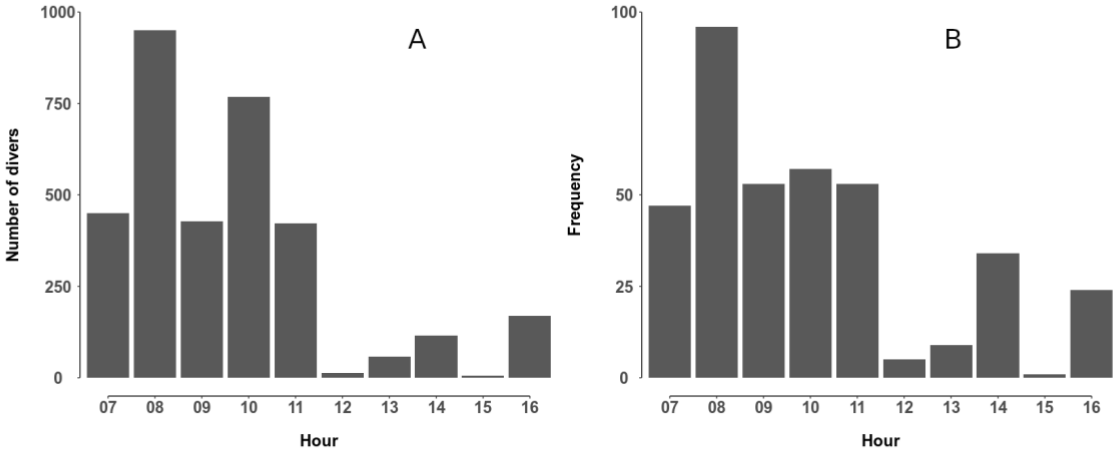

Figure 4.7. Illustration of both the number of divers per hour (A) as well as the hourly scuba-divers presence distribution (B). The hours in which the scuba-divers could not be present (i.e., from 17 00 to 06 00) in the study area were excluded.

3.1 Environmental drivers of the activity patterns in *E. marginatus*

The analysis of the AIC of the HMMs fitted to the activity data using different combinations of environmental variables suggests that, overall, all of them contributed to improve the performance of the HMMs (AIC_{Total}= 249,574) compared to those without any environmental variable (AIC_{Total}= 267,651). However, the importance of the different environmental variables to explain the activity patterns of the dusky grouper varied among individuals (Table 4.2). In general terms, storm conditions have a greater influence than other environmental variables on the HMMs (AIC_{Total} = 260,800), followed, in decreasing order of importance, by the time of the day (AIC_{Total} = 263,808), water temperature (AIC_{Total} = 263,814), current intensity (AIC_{Total} = 264,024), photoperiod (AIC_{Total} = 265,802), moon phase (AIC_{Total} = 267,068) and current direction (AIC_{Total} = 267,128). The assessment of the histograms and qq-plots of the pseudo-residuals of the final HMM suggested that our model fulfilled the normality assumption (Fig. 4.8 and Fig. 4.9). The estimates (± CI) of the HMM parameters are shown in Table 4.3. All the individuals remained most of the time in the active state, which is characterized by a higher mean (μ) activity and variance (σ) compared to the resting one (Table 4.3 and Fig. 4.4). The estimated μ and σ of the individuals 'D1' and 'H6' for the state resting were much higher than for the rest of individuals (Table 4.3), which was probably caused by the low frequency of low activity values (Fig. 4). Histograms and qq-plots of the pseudo-residuals of the HMMs fitted to the activity data using all the environmental co-variates suggested that our models fulfilled the normality assumption (Fig. 4.8 and Fig. 4.9).

Table 4.2 Hidden Markov Models (HMMs) comparison through the Akaike Information Criterion (AIC) for the assessment of the effect of the environmental co-variates in the activity patterns of *E. marginatus*. First, we compared the goodness-of-fit between different HMMs that used one environmental co-variate and the null-model (none co-variate was used in the HMM). Then, we compared the goodness-of-fit of different models to which the different co-variates were added one by one from the highest to the lowest AIC according to the previous step. Following the AIC criterion (AIC<3), we selected the model with the best combination of environmental co-variates (highlighted in bold).

Envir. Co-var.	D1	D2	D3	D4	D5	D6	D7	D8	H1	H2	H3	H4	H5	H6	Total
Null	24,636	19,472	16,324	14,531	22,394	22,101	10,459	8,049	18,053	21,598	22,714	21,561	22,291	23,468	267,651
Current direction	-8	0	-43	-33	-28	-63	-39	-36	-114	-63	-42	-16	-30	-8	-523
Current intensity	-280	-274	-186	-139	-816	-137	1	-193	-451	-213	-180	-60	-363	-336	-3,627
Moon	-14	-29	-13	-44	-66	-7	-110	-61	-18	-57	-43	-48	-25	-48	-583
Photoperiodism	-38	-98	-460	-156	-98	-164	-1	-93	-323	-63	-130	-106	-59	-60	-1,849
Storm	-342	-274	-197	-482	-1,071	-454	-58	-344	-1,079	-377	-694	-134	-573	-772	-6,851
Temperature	-121	-173	-1,373	-174	-93	-26	0	-204	-459	-146	-406	-329	-180	-153	-3,837
Time of day	-90	-125	-110	-70	-239	-408	-65	-74	-402	-202	-355	-253	-555	-895	-3,843
1+2	-404	-419	-1,399	-813	-1,351	-890	-183	-593	-1,444	-459	-1,192	-659	-1,200	-1,349	-12,355
1+2+3	-716	-751	-1,921	-895	-1,556	-972	-250	-652	-1,466	-670	-1,547	-987	-1,280	-1,366	-15,029
1+2+3+4	-828	-934	-2,014	-895	-1,710	-985	-277	-739	-1,861	-965	-1,550	-1,005	-1,675	-1,560	-16,998
1+2+3+4+5	**-880**	-1,105	-2,172	-946	-1,827	-998	-292	-796	-1,912	-1,004	-1,620	-1,012	-1,697	-1,563	-17,824
1+2+3+4+5+6	-877	**-1,135**	-2,192	-941	-1,887	-1,079	**-295**	-884	-1,953	-1,103	-1,674	**-1,070**	-1,737	-1,639	-18,466
1+2+3+4+5+6+7	-873	-1,132	**-2,205**	**-972**	**-1,921**	**-1,090**	-290	**-898**	**-2,010**	**-1,179**	**-1,695**	-1,072	**-1,765**	**-1,658**	**-18,760**

Table 4.3. Parameters of the Hidden Markov Model (HMM) fitted to the activity data of *E.marginatus* to test the effect of the marine current direction, marine current intensity, moon phase, photoperiodism, storm conditions, time of day and water temperature in its activity patterns. The intercept category for the categorical co-variates marine current direction and time of day was 'North' and 'Day', respectively. The table shows the estimated values (±Confidence Interval, CI) of the parameters for the activity (m s^{-2}) φ_t distribution per each state ('Active' and 'Resting) as well as the percentage of time (%) that the animals remained in each state. The parameters were estimated considering the mean value of the non-categorical co-variates. For the categorical co-variates marine current direction and time of day, the levels 'Day' and 'North' were used, respectively.

ID	Behavioural states					
	Active			Resting		
	Estimated parameters		%	Estimated parameters		%
	μ [±CI]	σ		μ [±CI]	σ	
D1	1.23 [1.20 - 1.26]	0,58	67,5	0.53 [0.47 - 0.59]	0,39	32,5
D2	0.96 [0.94 - 0.98]	0,59	83,9	0.17 [0.16 - 0.18]	0,07	16,1
D3	0.88 [0.87 - 0.90]	0,51	75,9	0.14 [0.13 - 0.14]	0,06	24,1
D4	1.21 [1.18 - 1.23]	0,55	73,2	0.23 [0.22 - 0.24]	0,12	26,8
D5	1.10 [1.08 - 1.13]	0,64	76	0.17 [0.17 - 0.18]	0,09	24
D6	0.98 [0.96 - 1.00]	0,62	77,5	0.14 [0.14 - 0.14]	0,06	22,5
D7	1.20 [1.17 - 1.24]	0,73	88,5	0.14 [0.13 - 0.14]	0,06	11,5
D8	0.84 [0.82 - 0.86]	0,48	71,1	0.12 [0.11 - 0.12]	0,05	28,9
H1	0.95 [0.93 - 0.96]	0,53	80,4	0.16 [0.15 - 0.16]	0,07	19,6
H2	0.97 [0.96 - 0.99]	0,54	84,9	0.15 [0.14 - 0.15]	0,07	15,1
H3	1.26 [1.24 - 1.28]	0,68	83,2	0.14 [0.13 - 0.14]	0,06	16,8
H4	0.99 [0.98 - 1.01]	0,61	79,1	0.15 [0.15 - 0.16]	0,07	20,9
H5	1.18 [1.16 - 1.20]	0,61	83,9	0.18 [0.18 - 0.19]	0,08	16,1
H6	1.13 [1.09 - 1.17]	0,60	62,7	0.42 [0.38 - 0.45]	0,24	37,3

Although the type and magnitude of the effect of the environmental variables (measured by the regression coefficients [β] in both the state-dependent distribution and transition probabilities) varied among individuals, some general trends were found (Fig. 4.10). Concerning seasonal patterns, the photoperiod had a significant effect either in terms of changes in activity intensity or state change probabilities on all individuals except D7 and H4 (i.e., in the 85% of the cases). However, the pattern was not consistent among individuals, existing positive, negative, or no significant photoperiod effects. Regarding the time of day effect in dusky grouper activity patterns, we found a trend of higher activity levels at night than at daytime since they were in the active state in 100% of the cases. Interestingly, that effect persisted for the resting state only for the individuals of the NTZ, for which in 83% of the cases (all except H6), the activity measured at the resting state was higher at night than during the daytime; for their part, no individual showed higher activities during the night than at daytime for the resting state for individuals from BD. Regarding the moon phase, in 79% of the individuals (except D1, D4 and D6), it caused some effect on the activity patterns of the dusky groupers. In terms of within-state activity level change, while there was a certain trend of activity decrease as the full moon approached when they were in the active state (present in 35% of the individuals, with no effect in the rest), there was no clear pattern when they were in the resting one. The effect of moon phase, nevertheless, was clearer at the level of the behavioural transitions: as the full moon approached, it increased the probabilities of changing to the resting state and decreased those of changing to the active state in 50 and 64 % of the individuals, respectively. Remarkably, the moon phase effect was clearer for individuals from the NTZ, for which 100% of individuals occurred some of the patterns described previously compared with 64% of individuals from BD. Concerning the water temperature effect, there were differences between individuals in line with the effect of other variables. However, in general terms (86% of the individuals), it was observed that as the temperature increased, there was an increase in the activity levels (either for the active or the resting state), an increase in the probability to transit to the active state, or a decrease in the likelihood to change to the resting one. The effect of storms, measured as the wave height, was one of the clearest in terms of among-individuals patterns consistency. As the wave height increased, the activity levels were higher for both the active and resting state in 93% of the individuals, with a decrease in the likelihood of them transiting to the resting state also in 93% of cases and no effect or a drop in the probabilities of changing to the active state in 100% of the individuals. As for the effect of current intensity, it was clearer in terms of within-state activity level change, with an increase in activity for either the active or the resting state in 71% of the individuals. In terms of behavioural state transition, the pattern was not that clear. Some individuals increased the likelihood of transiting to the resting state, others to the active one and no effect for the rest of them. Lastly, concerning the effect of current direction, it was clearer in terms of within-state activity level change, increasing the activity for either the active or resting state (or both) for southward marine currents in 64% of individuals. In terms of behavioural states transition, southward marine currents did not cause any effect in 64% of individuals, although a slight tendency existed to decrease the probabilities of transiting to the resting state (but only for 28% of individuals).

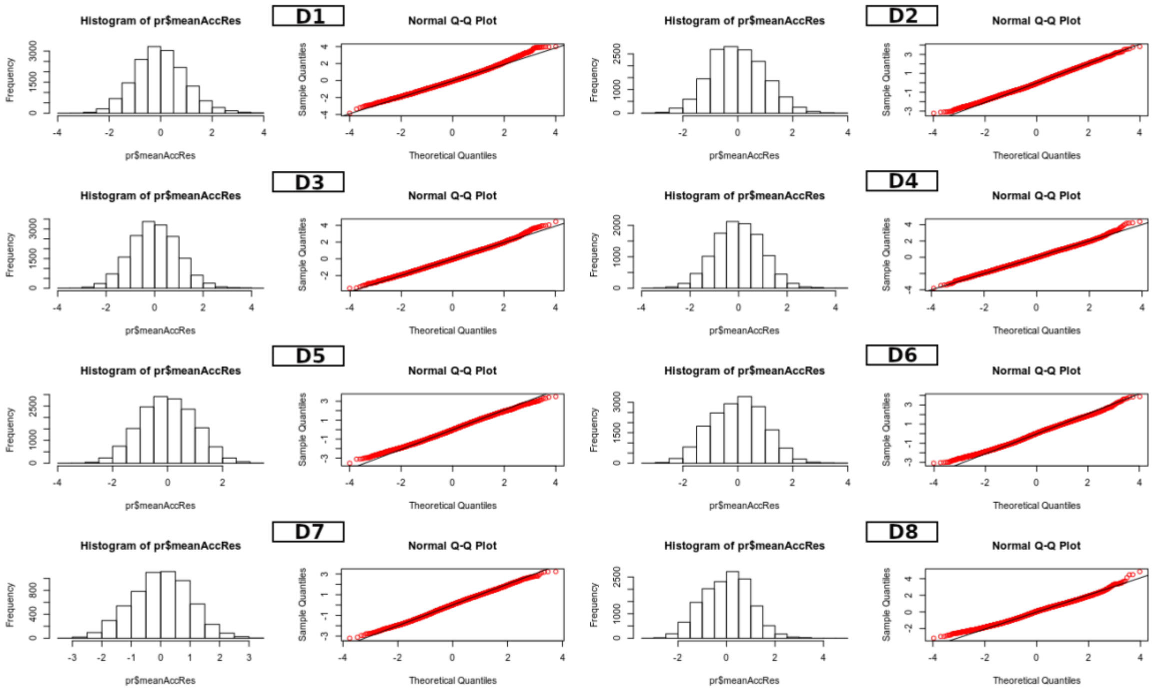

Figure 4.8. Histograms (left) and normal q-q plots (right) of the residuals of the HMM selected for each individual in the study site "Bajo de dentro" (BD) to assess the effect of environmental variables on the activity patterns of *Epinephelus marginatus*.

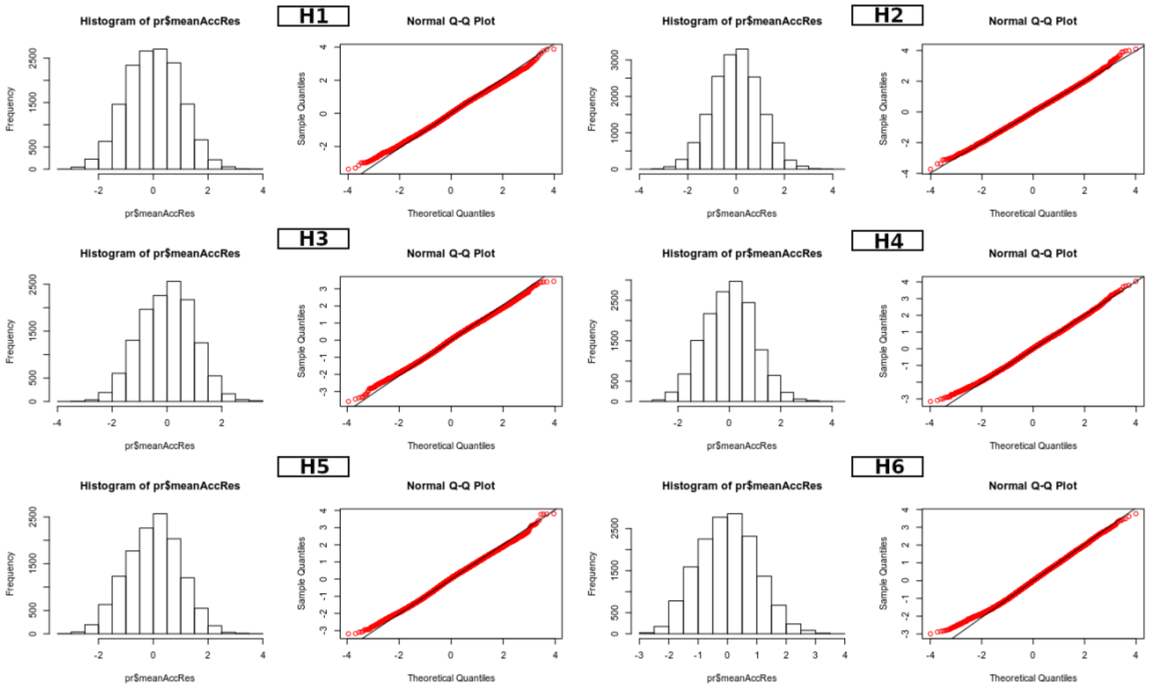

Figure 4.9. Histogram (left) and normal q-q plot (right) of the residuals of the HMM selected for each individual in the study site "Hormigón" (HG) to assess the effect of environmental variables on the activity patterns of *Epinephelus marginatus.*

3.2 Effects of the presence of SCUBA-divers in the activity patterns of *E. marginatus*

Histograms and qq-plots of the pseudo-residuals of the HMMs fitted to the activity data after selecting only data for the time of day ‘Day’ and including the divers' presence along with all the environmental variables (except time of the day) as co-variates suggested that our models fulfilled the normality assumption (Fig. 4.11 and Fig. 12). The analysis of the AIC of those models suggested that the presence of SCUBA-divers helped to improve the performance of the HMM for most (75%) individuals inhabiting the PPA where the SCUBA-diving activity is allowed (BD) but also for a good proportion (67%) of those present in the NTZ (HG) (Table 4.4). Overall, the HMMs which included the presence of SCUBA-divers as independent variable performed better (AIC_{Total} = 106,438) compared to those which considered only the environmental conditions as predictors of dusky grouper activity (AIC_{Total} = 106,726). Regarding the effect of interest (regression coefficient [β]), the type and magnitude of the effects of the presence of SCUBA-divers varied considerably among individuals (Fig. 4.13). In terms of within-state activity level change, in 50% of individuals from BD, there was an increase in their activities for either the active or resting state when SCUBA-divers were present, while in one individual (D4) its activity decreased, and in the case three individuals (38% of the total) the presence SCUBA-divers did not cause any effect. Surprisingly, in 33% of individuals from HG (the NTZ), we found that activity increased when the SCUBA-divers were present in the PPA. As for the effect of SCUBA-divers on the behavioural-states transition, there was considerable variability among individuals. In BD, some individuals (38%) reduced their behavioural transitions (either 'R -> A' or 'A -> R'), while most of them (63%) were not affected by the presence of SCUBA-divers. Contrary, in HG, there was only one individual (i.e., 17% cases) in which the presence of SCUBA-divers in the PPA affected its behavioural states transition, increasing the transitions to both the active and the resting state (i.e., more frequency of behavioural transitions).

Table 4.4 Hidden Markov Models (HMMs) comparison through the Akaike Information Criterion (AIC) for the assessment of the effect of scuba-divers presence on the activity behaviour of dusky grouper. We compared, per individual, the goodness-of-fit of a null model (m1) in which none variable was included as a predictor, a model in which only environmental co-variates were included (m2) and a model in which both the environmental and the scuba-divers presence co-variates were included (m3). Here we subset data for the time of day ‘Day’ since scuba-diving is forbidden at night at CPIHMFR. Thus, we included temperature, current intensity and direction, storms, moon and photoperiod, but exclude the time of day as co-variate in this analysis. The selected model, according to the AIC criterion (AIC<3), is highlighted in bold.

Covar.	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**
E.V.	20,675	13,749	10,312	12,715	16,169	14,335	6,125	3,041	11,842	17,486	15,096	14,803	13,379	15,883
E.V.+ Div	44	**-63**	**-20**	**-41**	**-19**	8	4	**-29**	2	**-17**	3	**-4**	**-5**	**-32**

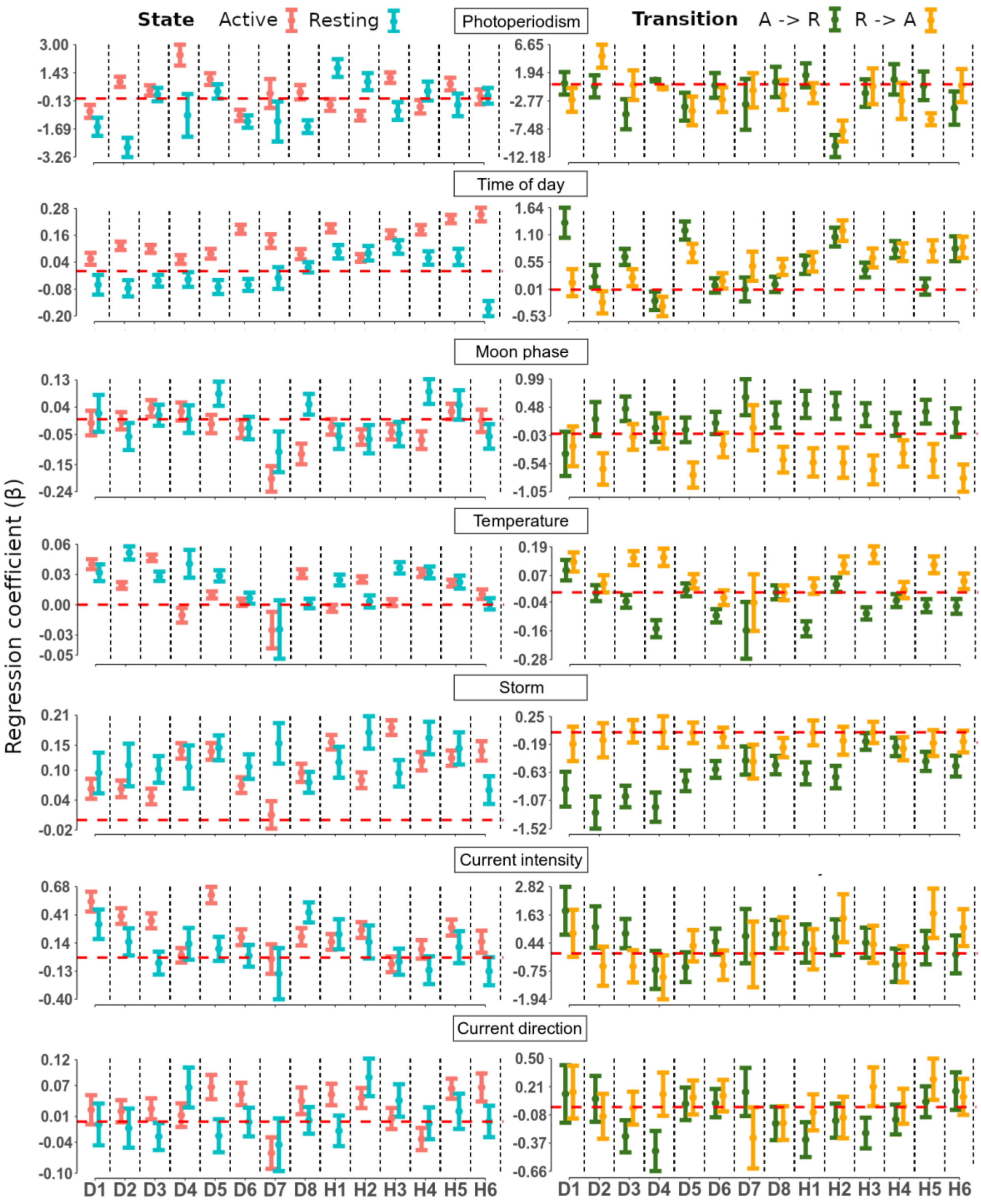

Figure 4.10. Effect of the environmental co-variates on the activity (m s^{-2}) patterns of *E. marginatus*. The plot shows the regression coefficients (β) estimate (± CI) for the state-dependent probability distributions 'Active' and 'Resting' (left column) and for the transition probabilities 'Active → Resting' and 'Resting → Active' (right column) obtained by fitting a Hidden Markov Model (HMM) to each individual. We considered a significant effect when the regression coefficients did not include the zero (denoted with a dashed red horizontal line).

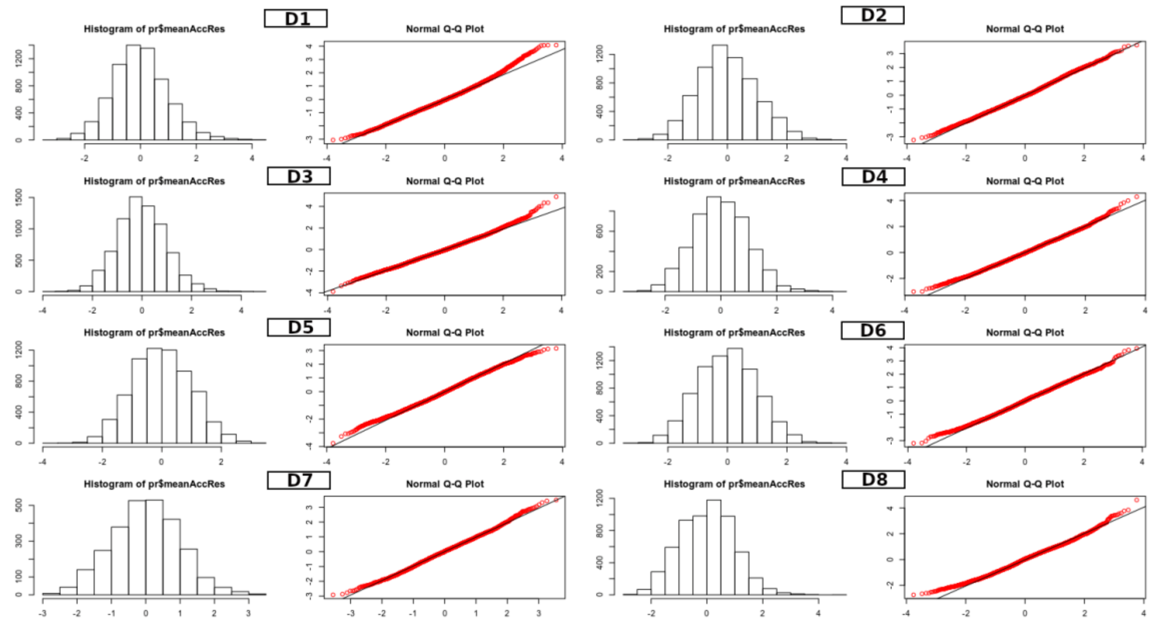

Figure 4.11. Histograms (left) and normal q-q plots (right) of the residuals of the HMM selected for each individual in the study site "Bajo de dentro" (BD) to assess the effect of scuba-divers-presence on the activity patterns of *Epinephelus marginatus*.

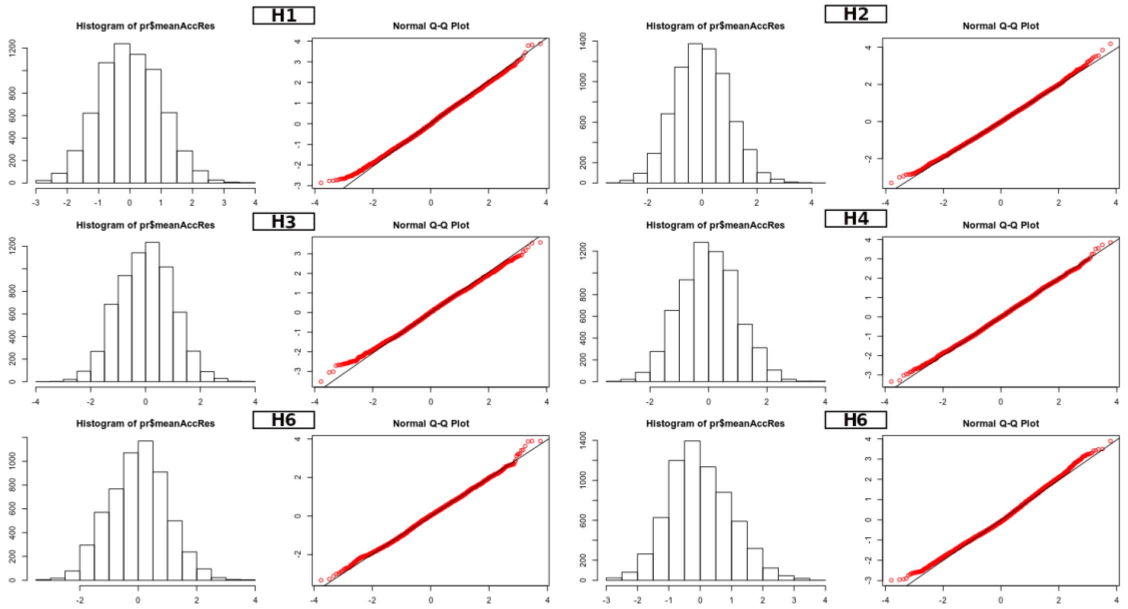

Figure 4.12. Histogram (left) and normal q-q plot (right) of the residuals of the HMM selected for each individual in the study site "Hormigón" (HG) to assess the effect of scuba-divers-presence on the activity patterns of *Epinephelus marginatus*.

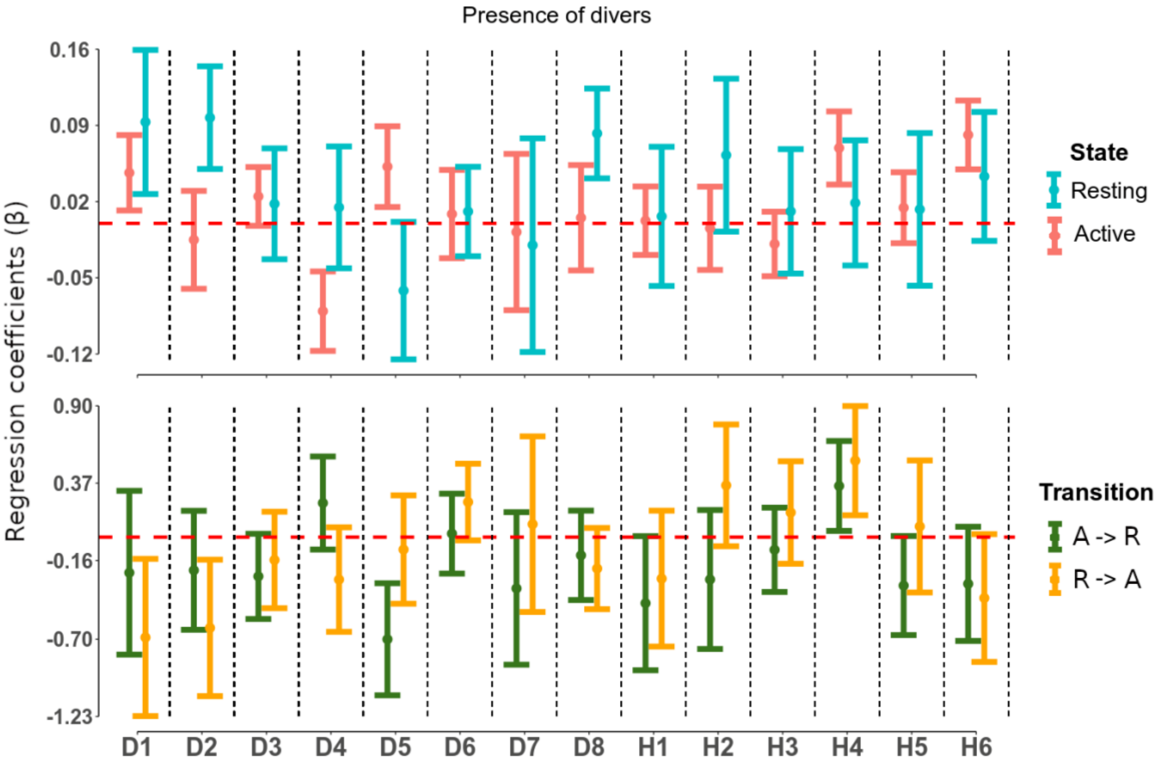

Figure 4.13. Effect of the presence of scuba-divers on the activity (m s^{-2}) patterns of *E. marginatus*. The plot shows the regression coefficients (β) estimate (± CI) for the state-dependent probability distributions 'Active' and 'Resting' (upper pannel) and for the transition probabilities 'Active → Resting' and 'Resting → Active' (lower pannel) obtained when fitting a Hidden Markov Model (HMM) to the activity data of *E. marginatus*. The marine current intensity, marine current direction, moon phase, photoperiodism, storm, temperature and presence of scuba-divers were included as co-variates. A horizontal red dashed line was added in the intercept equal to zero to identify when regression coefficients (± CI) were significantly distinct from zero. We only selected data for the time of day 'Day' and between the hours 07 00 and 16 00 following the existing legislation for recreational diving in the study area.

4. Discussion

Despite the interest in studying natural and human-induced drivers of aquatic animals behaviour in the context of global climate change (e.g., Sih *et al*. 2011, Nagelkerken & Munday 2016, van der Vinne *et al*. 2019) and a rapid increase in nature-based tourism (Geffroy *et al*. 2015, Wilson *et al*. 2020), information on these topics is still scarce, segmented and contradictory. This work separated the possible effect of SCUBA-diving from that of various environmental factors on the general activity patterns of *E. marginatus*, demonstrating the importance of the latter ones in determining the behaviour of this species. In addition to highlighting a series of clear behavioural patterns associated with variables such as the time of day, moon phase, temperature, and storms, this study showed the importance of a continuous revision of widely accepted assumptions that may be erroneous as a result of the use of non-validated ways of inferring activity (Watson & Harvey 2007, Chapter 3). Many species change their behaviour or physiology over the year as an adaptation to a changing environment (Falcón & Zohar 2018, Tsutsui & Ubuka 2018). These changes can be directly associated with the solar annual cycle or other closely related factors (e.g. temperature changes) (Falcón & Zohar 2018). Photoperiod is considered one of the main factors influencing fish breeding behaviour (Juntti & Fernald 2016, Falcón & Zohar 2018). In the case of dusky groupers, there is some controversy: while Hackradt (2012) and (Zabala *et al*. 1997b) found a clear shift in their activity level between summer (maximum) and winter (minimum), Koeck *et al*. (2014) encountered the opposite pattern. Our results, however, suggest seasonal cycles as an important but non-unidirectional (heterogeneity of strategies) selection force which would be advisable to incorporate in future aquatic animal behaviour studies.

As for the daily solar cycle, our results suggest an opposite pattern to those previously postulated, which defined dusky groupers as being mostly crepuscular, diurnal or indifferent to the time of the day (Gibran 2007, Pastor *et al*. 2009, Hackradt 2012, Azzurro *et al*. 2013, Koeck *et al*. 2014, Afonso *et al*. 2016). Here we found a clear trend to show higher activities at night than during the daytime, mostly when they were in an active state (which was most of the time). One plausible reason for *E. marginatus* to be nocturnal could be related to an increase in hunting success, as it would be more difficult for the prey to warn of their presence. Indeed, like groupers in general, the dusky groupers has relatively big-eyes, which is considered a typical adaptation of nocturnal animals (Gerkema *et al*. 2013, Wu *et al*. 2016, Potier *et al*. 2020). Another feasible reason might be that some of their preferred preys (e.g., *Octopus vulgaris*) have nocturnal behaviour (Kayes 1973, Brown *et al*. 2006), which would force them to increase their activity during these periods. Importantly, these results do not suggest that *E. marginatus* is a strictly nocturnal species, but it has a general trend of activity increase at night. Indeed, the results suggest they were active throughout the whole day, which might ensure a broader prey supply and is consistent with previous knowledge about its non-taxon specific diet (Condini *et al*. 2018). Moreover, the sharper nocturnal behaviour of dusky groupers from the NTZ (i.e., HG) could be related to ontogenetic shifts, habitat type, or even anthropogenic disturbances. On the one hand, the range of preys or the hunting success for *E. marginatus* might be size-related and force smaller individuals to increase their foraging when conditions are more optimal. Also, the type of habitat might determine food availability or light exposure. In this regard, the NTZ area has a more heterogeneous bottom and more available habitat (in terms of surface) at shallower depths than PPA (pers. obs.), which could explain, in the first place, why there was a trend of shallower depth distributions for individuals from NTZ. This habitat feature would also help to explain why individuals from NTZ had a sharper nocturnal behaviour since they are more exposed to light or have a higher range of food resources, increasing their foraging behaviour at night when conditions are more optimal. The last option would be related to the presence of SCUBA-divers. Dusky groupers in the NTZ showed a marked flight behaviour against SCUBA-divers presence (pers. obs.), with much larger escape distances than those in the PPA. This shyness could increase their activity at night when anthropic disturbances such as boat noise reduce abruptly, coinciding with previous literature about some species nocturnality behaviour as a response to human disturbances (Benítez-López 2018, Gaynor *et al*. 2018).

Despite the broad interest in the effect of the moon on fish behavioural patterns (e.g., Kneebone *et al*. 2018, Milardi *et al*. 2018, Brownscombe *et al*. 2019), its study in the dusky grouper is scarce and mainly focused on its impact on the breeding behaviour (Zabala *et al*. 1997a, Hereu *et al*. 2006). There is currently only one study evaluating the moon influence on general (i.e., non-reproductive) activity patterns of the dusky grouper (Hackradt 2012), without finding a clear relationship between moon phase and activity. Surprisingly, our results differed from those of Hackradt (2012), despite being conducted in the same area. In our case, the results evidenced the importance of the moon in the overall activity patterns, with a tendency to lower activities as the full moon approaches, an effect especially marked for individuals from the NTZ. This is consistent with previous studies about moon effect on spawning behaviour (Zabala *et al*. 1997a, Hereu *et al*. 2006) and our own results about the effects of the time of the day on the activity patterns of the dusky grouper, reinforcing the hypothesis about the importance of light on behaviour of the dusky grouper, with higher activities as its intensity decreases (i.e., at night and with a new moon). Regarding the sharper moon phase effect in individuals from the NTZ, the pattern was similar to that of the time of day, which would support the greater importance of light intensity for the activity pattern of individuals from the NTZ. This result might also indicate that the differences in behaviour between individuals from the NTZ and the PPA are not likely due to the human disturbance as stated previously since, in such as case, we would expect a non-significant effect of the moon phase given the low human disturbance at night. Thus, and following the reasoning described previously, the remaining most plausible explanation for the observed pattern might be associated with the individual size or/and the type of habitat.

Temperature is another environmental factor that has been widely studied concerning its effect on fish at both physiological and ecological levels (e.g., Biro *et al*. 2010, Breau *et al*. 2011, Brownscombe *et al*. 2019), being one of the most concerning issues associated with global climate change (e.g., Neuheimer *et al*. 2011, Wernberg *et al*. 2011, Holt & Jørgensen 2015, Ladich 2018). Temperature can condition aspects as diverse as digestion, somatic growth, reproduction, abundance or behaviour (e.g., Biro *et al*. 2010, Neuheimer *et al*. 2011, Schlaff *et al*. 2014, Waldock *et al*. 2019). In the case of dusky groupers, much of the studies so far have focused on assessing the relationship between temperature and reproductive behaviour, highlighting, in general terms, the importance of temperature as a trigger for spawning events (Condini *et al*. 2018). Only Hackradt (2012) evaluated the effects of temperature on dusky grouper activity patterns in non-reproductive periods, finding higher activities at higher temperatures. In line with it, we evidence a clear positive relationship between activity and temperature, although substantial individual variability was also observed, with some individuals whose activity is more thermo-dependent than others. Also, remarkably, this is the first work that separates this effect from the annual variation in activity, demonstrating the important role of temperature in activity patterns regardless of the time of year.

Storms are natural cyclical disturbances that can produce substantial changes in benthic communities (Posey *et al*. 1996, Madin & Connolly 2006, Teixidó *et al*. 2013). They also affect the behaviour of aquatic animals of various taxa (Udyawer *et al*. 2013, Schlaff *et al*. 2014), being a matter of concern due to the predictions of an increase in this type of phenomena with global climate change (e.g., Harley *et al*. 2006, Wernberg *et al*. 2011). However, most work using acoustic telemetry focused on assessing the spatial behaviour of animals associated with storms or uses not validated measures of activity such as the number of detections (see Chapter 3). In particular, information regarding the influence of storms on the activity patterns of dusky grouper is sparse and contradictory. While Hackradt (2012) observed a decline in the number of detections during storm periods, Koeck *et al*. (2014) observed that daily detection patterns were maintained even during storm periods. Nevertheless, none of these works used acoustic reference tags, which prevent us from stating that the observed patterns of detections were due to animal behaviour and not environmental variability (Payne *et al*. 2010). In our case, the activity data obtained is not affected by environmental variability, which allows us to affirm that storms events clearly affect the general activity patterns of dusky groupers, increasing notably their activity during these periods whenever they are outside their refuges. These results differed from those previously found, going in line with those of Hereu *et al*. (2006) about the importance of waves height in the spawning behaviour of dusky grouper, and underlining the importance of using adequate data when inferring activity derived from acoustic telemetry devices.

Marine currents are another environmental factor that affects benthic communities and the behaviour of the aquatic species that inhabit them (Montgomery *et al*. 2000, Metcalfe *et al*. 2006). However, most studies focus on assessing their effects in terms of movement (e.g., Metcalfe *et al*. 2006, Childs *et al*. 2008, Kelly & Klimley 2012, Schlaff *et al*. 2014), paying little attention to their effects on non-spatial patterns such as temporal activity patterns. In general, it is hypothesized that aquatic animals use the water currents as an efficient energy strategy (Brodersen *et al*. 2008, Kelly & Klimley 2012, Schlaff *et al*. 2014). Information on the possible effect of water currents on non-highly mobile species like dusky grouper is scarce. In Hereu *et al*. (2006), no significant relationship between reproductive behaviour and marine currents was found. In Hackradt (2012), while water current was found to be one of the leading environmental factors that modulated the number of detections for *Epinephelus costae*, it did not practically help to explain those of *E. marginatus*. Our work, however, evidenced the influence, in general terms, of marine currents on the temporal activity patterns of dusky grouper, although with considerable variability among individual behavioural strategies. This would be supported by the differences between Hereu *et al*. (2006) and Hackradt (2012) regarding the response of dusky groupers to water currents. While Hereu *et al*. (2006) noted that males sheltered during periods of strong marine currents, Hackradt (2012) observed that individuals positioned themselves in the water column facing the water currents, which is evidence that the behavioural response is probably not homogeneous among individuals or even over time, and could also be site-specific. Thus, we believe that this study interestingly suggests that marine currents can modulate not only the spatial behaviour of the species but also their activity rhythms.

Although interest in studying the impact of recreational diving on aquatic animals is not recent, most work is based on methodologies that do not consider long-term individuals tracking (Smith *et al*. 2014, Titus *et al*. 2015, Valerio *et al*. 2019). To our knowledge, this work is the second to address this issue using individuals tracking methods (the first was Bradley *et al*. 2017), and the first to use activity data. The large variability in the type and magnitude of effects, if any, lead us to conclude that there is no clear SCUBA-diving impact on *E. marginatus* activity patterns. Also, the striking similarity of the results between individuals in the area with and without diving, make us take these results with caution. On the one hand, they could evidence that SCUBA-diving activity associated noise might affect fishes that are not directly in contact with divers, since the distance between the two areas (1.2 km) would allow the sound of the engines of these boats (with powers above 100 hp generally) attain the NTZ with relative intensity (John C. Montgomery; pers. comm.) (Kaplan & Mooney 2015, McCormick *et al*. 2019). The disparity of results among studies (e.g., Cubero-Pardo *et al*. 2011, Smith *et al*. 2014, Titus *et al*. 2015, Bradley *et al*. 2017, Valerio *et al*. 2019), along with the response variability among individuals analysed here for different environmental variables, augur the possible heterogeneity of responses even among individuals of the same species. Nonetheless, interpretations must be made cautiously, since factors not considered here, such as the diver behaviour or the position of both the animal and the diver, which determines the establishment of visual contact between them, could be crucial aspects when evaluating it (Cubero-Pardo *et al*. 2011). To our knowledge, this is the first study that analyses the effect of the SCUBA-diving activity on temporal activity patterns, reporting, importantly (Bateman & Fleming 2017), its little or no effect on the high trophic-level predator species *E. marginatus*.

In short, the present study is the first to simultaneously evaluate the effect of a range of environmental variables and the SCUBA-diving activity on activity patterns, using a control zone and a statistical technique of increasing interest (i.e., HMM), since it allows to consider the transitions between behavioural states. Also, remarkably, this study covered non-breeding periods of the species, which account for most of their time (Marsden *et al*. 2021). Here we evidence the potential importance of all environmental variables evaluated in the activity patterns of *E. marginatus*, separating, for example, the effect of temperature from seasonal cycles, two variables usually not considered separately (e.g., Hackradt 2012, Koeck *et al*. 2014). However, there were substantial differences among environmental variables as for their influence on dusky grouper activity patterns, with clear heterogeneity among individuals regarding their responses. The environmental variables for which a relatively clear behavioural pattern was found were the time of day, the moon phase, the temperature and the occurrence of storms. Given the discrepancies of our results with previous widely accepted statements, we believe this work underlines the complexity of the study of drivers of the behaviour of aquatic animals and the need to be very cautious in the conclusions drawn since they might be crucial from a management point of view. Previous works that assessed the activity patterns of dusky grouper had a series of methodological limitations that could call into question their conclusions. Some of them used visual methods (Zabala *et al*. 1997a, b, Gibran 2007, Azzurro *et al*. 2013), which involve the non-tracking of individuals and whose performance in inferring activity, besides not being validated, is questionable (Watson & Harvey 2007, Tuyttens *et al*. 2014). Others used techniques based on passive tracking of individuals (acoustic telemetry) (Pastor *et al*. 2009, Hackradt 2012, Koeck *et al*. 2014, Afonso *et al*. 2016), but used a type of variable that is not appropriate for inferring activity (Chapter 3). Moreover, some of them used a too small number of individuals (Koeck *et al*. 2014), or environmental data of very low spatial resolution (e.g., Hackardt 2012 used temperature data from a sensor located 29 km from the study area and placed at 3 m depth). For all these reasons, we strongly believe that we present here the most reliable results to date on the activity patterns of *E. marginatus*, understanding that they can be of great use in improving the sustainable management of this emblematic species; examples of management measures to be adopted are limitation or, conversely, permission to fish during known periods of high activity for dusky grouper depending on what the main management objective is - to conserve stocks or to boost the traditional small-scale fishing sector, respectively.

Chapter V

General Discussion

1. Contribution of the thesis to the study of aquatic animal's activity behaviour

The study of animal activity patterns in the aquatic environment using acoustic telemetry poses some problems. On the one hand, studies typically use indirect activity measures that have not been yet validated as activity proxies, such as the number of detections, the depth range, or the distances travelled (Alós *et al.* 2012, Skerritt *et al.* 2015, Christoffersen *et al.* 2019). On the other hand, although acceleration data are starting to be used as an indirect activity measure whose usefulness has been widely validated with the use of conventional accelerometers (Wilson *et al.* 2019), its performance related to the constraints of acoustic telemetry has not been tested to now. All this inevitably leads to the question of whether the data obtained from acoustic telemetry are appropriate for inferring aquatic animal activity. Answering this question requires the simultaneous use of acoustic transmitters incorporating activity sensors (i.e., accelerometers) and conventional (i.e., non-acoustic) accelerometers. This methodological approach has the difficulty of having to synchronise the two devices, which constitutes a challenge given the inherent drift of any clock (Packard 1997, Zhou *et al.* 2008, Castillo-Secilla *et al.* 2013), which in turn depends on multiple factors, such as the material, the age of the clocks or the physical conditions to which they are subjected (Tirado-Andrés *et al.* 2019). This is a major issue for aquatic species that move continuously along the water column since the devices placed in them suffer continuous and unpredictable changes in pressure and temperature. In this sense, throughout the different chapters of this thesis, we managed to answer the question of the usefulness of different types of data from acoustic telemetry devices in inferring activity in the dusky grouper, in order to finally evaluate the effects of a series of environmental variables and the presence of SCUBA divers on the activity patterns of this emblematic fish species (Condini *et al.* 2018). Importantly, by evaluating the usefulness of different acoustic telemetry data types, we were able to discuss possible reasons for discrepancies in the results between our study and previous works regarding dusky grouper activity patterns.

The existing literature on the correction of clocks drifts is scarce and complex to apply (Schenato & Fiorentin 2011, Jiang *et al.* 2018, Coviello *et al.* 2020). Moreover, in certain fields, such as studying aquatic animal behaviour, this information may not be convenient for previously mentioned reasons. The first chapter of the thesis contributes substantially to overcoming this problem, as it proposes a synchronisation method that does not require knowledge of the characteristics of the device or the type of environmental conditions to which they are subjected when recording. This method even makes it possible to synchronise devices that were incorrectly synchronised from the beginning or that, for some reason, they desynchronised throughout the study. Chapter 2 demonstrates that the assumption on which the proposed method is based is valid and can be useful in different scenarios. As demonstrated in Chapter 3, the characteristics of acoustic telemetry technology trigger the risk of its performance to vary depending on individuals or species behaviour, making it advisable to carry out similar studies to Chapter 2 but in other species. To this end, it was important to have a method for synchronising devices such as the one proposed in Chapter 2, as it should be noted that desynchronisation between pairs of devices starts from the time-setting moment, which in the field of acoustic telemetry is usually several days before the devices are deployed in the field. Furthermore, this type of studies should be accomplished over several days, accumulating a certain time drift between devices. In this regard, this synchronisation method allows synchronising the devices every so often (for example, once a day).

A relatively large number of works (compared to other species) has studied the activity patterns of dusky groupers (Zabala *et al.* 1997a, b, Gibran 2007, Pastor *et al.* 2009, Hackradt 2012, Azzurro *et al.* 2013, Koeck *et al.* 2014, Afonso *et al.* 2016). However, the type of methodologies used generates doubts about the reliability of the conclusions drawn, as either visual methods or non-validated data from acoustic telemetry were used for inferring activity. In this regard, Chapter 3 sheds light on the validity of different types of acoustic telemetry-derived data for inferring activity in the dusky grouper, highlighting, among others, that only acceleration data are suitable for this purpose. This has important implications, as none of the work to date on dusky grouper activity patterns has used this type of data. However, we believe that this work is valuable for some other reasons. First, it proposes a new formula for estimating activity with acoustic accelerometers that is insensitive to the sampling window size used, which is crucial as it is hoped that with the increase in the number of papers using this type of data (Cooke *et al.* 2016), comparisons between studies will also increase. Second, it can point to future research lines to improve the performance of acoustic accelerometers. Third, it validates the averaging of raw activity data over longer time windows. Fourth, it highlights the need for caution when interpreting activity data due to both the risk of sampling-bias in data collection and the reduced amount of data (intermittency), leading to a limited ability to detect certain effects such as time of day.

Knowledge about the activity patterns of aquatic animals in general, and dusky grouper in particular, is scarce and questioned for the reasons given above. This is relevant because of the ecological and socio-economic importance of the dusky grouper (Condini *et al.* 2018). For this reason, in Chapter 4 we study the effect of various environmental factors on the activity patterns of dusky grouper, including the impact of SCUBA-diving, an activity that is one of the main economic drivers in the coastal area. This study is the most reliable to date in terms of dusky grouper activity patterns not only because it uses acceleration data, but also because it uses a control area and uses a novel statistical technique (Hidden Markov Models) that allows us to define behaviours as well as contemplate effects on the transition probability among behaviours (Smokorowski *et al.* 2017, McClintock *et al.* 2018). As a result, we were able to demonstrate that dusky grouper activity patterns are modulated by numerous environmental factors, but also that there is a large heterogeneity among individuals of responses to different types of perturbations. In general terms, the clear effect of environmental factors such as storms, time of day, temperatures or the moon on dusky grouper activity patterns is noteworthy. Regarding the importance of currents (both intensity and direction), photoperiod, or the presence of SCUBA divers, there was no clear response patterns, although it was evident that they can influence dusky grouper activity. As noted in Chapter 4, some of the conclusions drawn in this chapter are not in line with previous studies. For example, there was no consensus about the effect of storms on activity patterns of dusky groupers (Hereu *et al.* 2006, Hackrad 2012, Koeck *et al.* 2014); it was assumed that they were diurnal or twilight (Gibran 2007, Pastor *et al.* 2009, Hackradt 2012, Azzurro *et al.* 2013, Koeck *et al.* 2014, Afonso *et al.* 2016), or that the moon had no clear effect (Hackradt 2012). Therefore, the thesis evidenced the possible limitations of previous works when studying activity patterns in aquatic animals, highlighting the need to further research in these lines.

In summary, this thesis made a major contribution to a research line claimed recently: understanding acoustic telemetry data to interpret the results properly (Brownscombe *et al.* 2019). Specifically, we focused on how to interpret activity patterns. On the one hand, it proposed both a method for synchronising devices and a methodology to evaluate acoustic telemetry performance in inferring activity, which is necessary because different species behave differently and occupy habitats with very different acoustic properties. On the other hand, through a better understanding of acoustic telemetry data, it provides new ecological information on a species such as groupers, which is of great scientific, socio-economic and conservation interest (Condini *et al.* 2018), and which could be useful to design effective management measures that combine their conservation with their enjoyment by nature-based tourist and their exploitation by the fishing sector (professional or recreational).

2. Future perspectives

Although this thesis has contributed to the study of activity patterns in aquatic animals, there are still numerous issues addressed to be further investigated. In Chapter 2, regarding the method for synchronising devices that simultaneously recorded data with some natural correlation, its performance was not evaluated, among other cases, when the devices recorded data of different nature. Thus, although we hypothesised that the proposed method should also be valid in this last scenario as long as the conditions of simultaneous data and some natural correlation between device data are met, this has to be still tested and validated. An example could be synchronising an atmospheric temperature sensor and a light-intensity one, given the expected correlation between the two variables. Regardless of that, it would be highly advisable to carry out further tests and continue to study which aspects ensure that the synchronisation is as accurate as possible.

In Chapter 3, several research lines about acoustic telemetry data performance assessment for inferring aquatic animal activity remained open. The development of similar research in other species and other types of habitats, or comparisons between acoustic and conventional accelerometers under lab-controlled conditions would be highly advisable to properly interpret acoustic telemetry data. Lastly, although Chapter 4 developed one of the best sampling designs applicable to date to study activity patterns (Zucchini *et al.* 2016, Smokorowski *et al.* 2017), some aspects could be improved. On the one hand, the spatial resolution of variables such as the marine currents (derived from regional models) or the presence of SCUBA divers could be improved, which would perhaps help to discern whether they really have a clear effect on the dusky grouper activity patterns. For example, the diver's position concerning the seamount or islet is important, as it determines the actual interaction between the diver and the animal (Cubero-Pardo *et al.* 2011). This information, nonetheless, is not available as the diver's path is unknown until they are in the water, and they check water conditions (mainly marine currents and turbidity). Therefore, although this chapter contributed to understanding dusky grouper activity patterns, there are still aspects like those mentioned above or the inclusion of other variables (e.g., turbidity) that could improve our interpretation of dusky grouper activity pattern drivers.

Beyond the topics strictly addressed in this thesis, the incorporation of accelerometers in acoustic transmitters offer a range of possibilities in fields such as ecology or evolution (Cooke *et al.* 2016, Wilson *et al.* 2019), especially after our Chapter 3, that shows the non-validity of some variables historically used in acoustic telemetry to infer activity. Questions such as when, how, and where species consume energy or how they do it are important to understand the distribution of species by interpreting the adaptation and evolutionary processes they have experienced. In this respect, accelerometer data have proven to be very useful in answering many of these questions. For example, thanks to the close relationship between energy cost and acceleration in fish, accelerometers provide a crucial breakthrough in the field of bioenergetics (i.e. energy consumed = Metabolism + Waste + Growth), a foundational concept in fish biology (Cooke *et al.* 2016). But accelerometers, in addition to helping to answer questions related to how much and when, are of great use for answering the ‘how’ question, since from these data body positions and thus specific behaviours can be derived (e.g., Beltramino *et al.* 2019). However, the usefulness of the data provided by accelerometers goes far beyond those merely associated with bioenergetic aspects. Other types of questions such as the environmental or human-induced effects on the behaviour of animals can be addressed with this technology, questions that are crucial from the point of view of sustainability as they would help to take management measures that reconcile the human use of ecosystems with their conservation (Wilson *et al.* 2020). An example derived from the development of this thesis would be to investigate the possible impact of ship noise on fish behaviour, a subject of historical interest (e.g., Montgomery *et al.* 2017, McCormick *et al.* 2019, Barcelo-Serra *et al.* 2020) but little developed to date due to the technological limitations to address it. In the case of dusky groupers, if it were proven that noise from human activities from outside the NTZ affects individuals from this presence restricted area, it would be of interest as this could have implications from the management of marine protected areas. The use of data obtained by accelerometers onboard acoustic transmitters, however, has its peculiarities. On the one hand, its main disadvantage is that by calculating a single activity value for relatively large time windows, in addition to losing temporal resolution (Cooke *et al.* 2016), it is not possible to infer specific body positions or behaviours. In contrast, the main advantage is the possibility to spatially describe the activity behaviour of tagged individuals (Cooke *et al.* 2016), as acoustic telemetry, by appropriate design, allows positioning of acoustic detections. This is of enormous value because it would allow answering important questions such as where reproductive aggregations of species like grouper occur, and what are the energetic costs of such events; what could be the impact of global climate change in terms of distribution and abundance of species associated with changes in the energy balances of food webs, or how marine protected areas can help to mitigate these or other impacts associated with human activity, thanks to the greater resilience of MPAs as a result of their good conservation status. In short, the incorporation of accelerometers in acoustic transmitters opens up tremendously interesting and complementary lines of research to others that can be addressed with the use of conventional accelerometers, generating very promising perspectives for progress in the acquisition of basic and applicable knowledge for the sustainability of human life on planet Earth.

General Conclusions

Chapter 2:

1. Overall, the highest correlation degree between pairs of accelerometers or temperature sensors data that were recorded simultaneously throughout time occurs when both devices are synchronised. Based on this hypothesis, we proposed and test an operational synchronization method of pairs of data-log devices. The proposed synchronisation method was effective and robust in synchronising data from pairs of devices that recorded acceleration or temperature data simultaneously over time, with temporal autocorrelation as the most critical aspect to consider in achieving reliable synchronisations.

2. Different pairs of X16-mini/V13AP showed distinct desynchronisation times, which proved that synchronising pairs of devices just considering theoretical clocks drift might be inappropriate, at least for devices subjected to strong and unpredictable changes in temperature and pressure conditions.

3. The proposed synchronisation method proved useful in developing methodological studies that assess the performance of acoustic telemetry data for inferring activity.

Chapter 3:

4. Activity data from accelerometers onboard acoustic transmitters placed inside the animals are valid for inferring activity regardless of accelerometers have narrow acceleration ranges (3G) and low recording frequencies (5 Hz). The V13AP explained 89% variability of the X16-mini, a conventional accelerometer with an acceleration range five times greater than that for the V13AP (i.e., 16 G), recorded at 12 Hz and whose external position in the animals' body allowed them to be stable.

5. Raw activity data averaged over larger time windows than those in which activity was recorded (i.e., 57 s) shown to be valid for inferring activity in dusky grouper. Nevertheless, the greater or lesser accuracy in estimating activity depended on the time bin size and/or the number of activity records for that time bin. The choice of the time bin proved to be a trade-off between small time windows (e.g. 6 min) that

increase the risk of underestimating real activity and large ones (e.g. 120 min) that reduce the activity temporal resolution. A minimum number of activity records per time bin could be appropriate to maximise the probability of estimating activity accurately whenever using time bins long enough to assign a minimum threshold for the number of activity records.

6. Results from previous studies regarding dusky grouper activity patterns should be taken with caution as they were developed with unvalidated methods (e.g., visual) or data that have proven to be inappropriate for inferring activity. In acoustic telemetry, activity data from accelerometers are the only valid method for inferring activity in the dusky grouper. The prediction power of the variables ‘number of detections’ and ‘depth-range’ was always very poor, with percentages of X16-mini variance explained below 5% and 25%, respectively.

8. There is a risk of sampling-bias in the collection of activity data when using acoustic telemetry, a method whose data collection is dependent on the position of the animal. In dusky grouper, in all the cases in which there was a sampling-bias, it was in the same sense: a slight tendency to higher activities for periods in which they are audible, suggesting that with acoustic telemetry, we might obtain a proportion of high activity values greater than the real one. However, the occurrence and magnitude of this sampling-bias vary even among individuals of the same species, suggesting caution when interpreting the data.

9. Acoustic telemetry data were shown to have a proper but limited ability to predict the effect of ‘time of day’ on dusky grouper activity patterns. Data from acoustic transmitters allowed to identify this effect only for those individuals in which it was sharper, underlining the need to interpret with caution this type of data, which are intermittent, come from accelerometers with additional restrictions to the conventional ones, and depend on environmental conditions as well as the individual behaviour.

Chapter 4:

10. Dusky groupers showed great response heterogeneity to multiple environmental variables, including storms, time of day, temperature, moon phase, photoperiod, and marine currents. Overall, they increased their activity as storms intensity or temperatures increased, while decreasing in high light conditions (i.e., during the daytime or as the full moon approached). In contrast, although most of the individuals seemed to respond to the environmental variables ‘photoperiod’, ‘current intensity’ and ‘current direction’, no clear overall pattern was found.

11. The sharper effect of the time of day and moon phase for individuals from the NTZ compared to individuals from the PPA is evidence of the complexity of studying animal behaviour, as it is likely that factors such as the size of individuals or habitat type also affect the observed behaviours.

12. Although some individuals from the NTZ and the PPA reacted to the presence of SCUBA-divers in terms of activity, there was no evidence of a common dusky groupers behavioural response. The fact that some individual from the PPA were affected by the presence of SCUBA-divers in the NTZ suggests that boat engine noise could be a plausible source of disturbance for fish, that should be further investigated.

13. The discrepancies between some of the results of Chapter 4 and those of previous works concerning the effects of the variables 'storms', 'time of day' and 'moon phase' corroborate the possible limitations of the conclusions from previous studies because of the type of data used. Therefore, it would be advisable to review the conclusions concerning activity patterns drawn thought acoustic telemetry without using acceleration data as the activity proxy.

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Appendices

Appendix A. R-code for accelerometers synchronisation. The parts of the code underlined in blue indicate where the code needs to be configured to correctly run the synchronisation code, with small clarifications on these parts of the code in green notes.

```
### Global options ####
options("digits.secs" = 3)
set.seed(1)

### Libraries ####
library(dplyr)
library(lubridate)
library(data.table)
library(ggplot2)

### Comments ####

# Colum names for data of devices:
        # Date and time → Datetime
        # Acceleration in X-axis → AccX
        # Acceleration in Y-axis → AccY
        # Acceleration in Z-axis → AccZ

### Data load ####
  # Device 1 ####
reference_device <- readRDS("…")  # Directory where data for the reference device is located.

  # Device 2 ####
settable_device <- readRDS("...")  # Directory where data for the settable device is located.

### Results directory and object ####
```

```
results_directory <- "…"        # Directory where we want to save the files generated for the sync process.
final_results <- data.frame()

### Function declaration ####
data_domain <- function(tipo_analisis){
  #' Function to preprocess the original database depending the input data
  #'
  #' @description P
  #'
  #' @param tipo_analisis character: "acc", "temp"
  #' @usage data_domain("acc")

  if (tipo_analisis == "acc"){
    names(reference_device) <<- c("Datetime", "AccX", "AccY", "AccZ")
    names(settable_device) <<- c("Datetime", "AccX", "AccY", "AccZ")

    ### 'Datetime'  type ####
    reference_device$Datetime <<- as.POSIXct(reference_device$Datetime, format = "%Y-%m-%d %H:%M:%0S", tz = "UTC")
    settable_device$Datetime <<- as.POSIXct(settable_device$Datetime, format = "%Y-%m-%d %H:%M:%0S", tz = "UTC")

    # Results directory
    dir.create(file.path(paste0(results_directory, tipo_analisis)), showWarnings = TRUE)
  }
}
sync_acc <- function(data, originT, lowerW, upperW, delay){
  #' Function to create a vector column with new time activities interval in which VeDBA values must be calculated
  #'
  #' @description Calcultate aactivity intervals in which compute VeDBA values
  #'
```

```
  #' @param originT integer The time (in minutes) that must be added to the first
timestamp.
  #' @param lowerW integer The lower (in seconds) time window value
  #' @param upperW integer The upper (in seconds) time window value
  #' @param delay integer The delay. Activity duration window (in minutes)
  #' @usage sync_acc(originT, lowerW, upperW, delay)
  #' @return A dataframe.
  #' @details The input must be four numeric values
  #'

  # Re-calculated first timestamp of the data, once we have added the originT.
  # This is perfom to ensure that time can be substracted in further steps when
comparing interval windows before t0
  # data must be a dataframe
  names(data)[1] <- "Datetime"
  first_value <- data[(which(data$Datetime > data[1,]$Datetime + minutes(originT))),
c("Datetime")][1]

  # Last timestamp of our object
  last_value <- data[nrow(data), c("Datetime")]

  # Updated timestamp value. The first value of "updated_value" is "first_value", then
it is re-calculated
  updated_value <- first_value

  # A dataframe in which the mid-results are stored
  df <- data.frame(Date = as.POSIXct(character()),
                   Sampling_delay = integer(),
                   Initial_activity = as.POSIXct(character()),
                   Final_activity = as.POSIXct(character()))

  # Benchmark
  inicio <- Sys.time()
```

```
  # Loop that ends once the "updated_value" is after the "last_value"
  while(last_value > updated_value){
    # A row with the results are computed.
    # Data frame structure must be used
    result <- as.data.frame(updated_value) %>%
      # We randomly select a value between the lower and upper window interval
      mutate(Sampling_delay = sample(lowerW:upperW, 1, replace = TRUE),
             Initial_activity = updated_value + seconds(Sampling_delay),
             Final_activity = Initial_activity + seconds(delay))

    # Result row is added to the declared df
    df <- rbind(df, result)

    # "updated_value" is updated
    updated_value <- result$Final_activity
  }

  # Benchmark
  fin <- Sys.time()
  # Execution duration
  print(fin-inicio)

  # Return the dataframe
  return(df)
}

### Setting parameters ####
sampling_frequency <- 12     # Number of records per unit of time (here is ‘second’).
threshold <- 0.9     # Minimum proportion of samples per unit of time to consider that sampling window.
sampling_window_size <- 20    # Size of the sampling window (here 20 seconds).
n_iterations <- c(1:15) # Number of iterations per combination of configurable parameters.
```

```
time_delay <- seq(from = -100, to = 50, by = 10)    # TDR ("from" and "to") and TDRes ("by").
origin_displacement <- 15 # Time lapse between the origin and the time in which we start to sample data.
minimum_sampling_delay <- 60    # Mimimum time distance between consecutive samples.
maximum_sampling_delay <- 180    # Maximum time distance between consecutive samples.

### Statistical method to measure correlation strength ####
# Options: "spearman", "pearson" or "lm" (linear-model)

correlation_method <- "spearman"

approach <- "acc"

if (approach == "acc"){
  data_domain("acc")
  n_min <- sampling_frequency * sampling_window_size * threshold
  # Acelerómetro
  for(cases in n_iterations){
    print(paste0("Iteration number: ", cases))
    # We run the function
    time_interval <- sync_acc(data = reference_device, originT = origin_displacement, lowerW = minimum_sampling_delay, upperW = maximum_sampling_delay, delay = sampling_window_size)

    # Only Initial_activity values are needed. We select and rename it to "Datetime"
    time_interval <- time_interval %>%
      select(Datetime = Initial_activity)

    # Tibble are converted to Data_table structure
    # Here the delay must be specified again
```

```
  setDT(time_interval)[, Datetime := as.POSIXct(Datetime, format = fmt, tz =
"UTC")][,c("start", "end") := .(Datetime, Datetime + sampling_window_size)]
  setDT(reference_device)[, Datetime := as.POSIXct(Datetime, format = fmt, tz =
"UTC")]

  # ADD COMMENT
  time_interval <- reference_device[time_interval, on = .(Datetime >= start,
Datetime <= end),
                   by = .EACHI, .(
                    Datetime = i.Datetime, .N,
                    # Compute VeDBA values for first device
                    VeDBA.reference_device = if (.N < n_min) NA_real_ else
sum(sqrt(AccX ^ 2 + AccY ^ 2 + AccZ ^ 2)) / .N
                   )][,(2L:3L) := NULL][]

  # In case all N value es lower than the minimun N, we stop the execution
  if(all(time_interval$N<n_min)){
   print("N es lower than n_min")
   break
  }
  else{
   # Remove observations in which less than n_min actity points are used
   time_interval <- time_interval[time_interval$N>=n_min,]
   # ADD COMMENT
   for (i in time_delay){
    objeto_auxiliar <- settable_device
    objeto_auxiliar$Datetime <- objeto_auxiliar$Datetime + i

    setDT(time_interval)[, Datetime := as.POSIXct(Datetime, format = fmt, tz =
"UTC")][,c("start", "end") := .(Datetime, Datetime + sampling_window_size)] #
"DetectionsHAleAcc" contiene los tiempos de toma de actividad para los V13AP.
Calcula actividad para 57 segundos. También contiene el valor de actividad para
esos 57 segundos del V13AP
```

```
    setDT(objeto_auxiliar)[, Datetime := as.POSIXct(Datetime, format = fmt, tz =
"UTC")]   # "objeto_auxiliar" son los datos del X16-mini con un "delay" determinado
(que va desde -180 a +180)

    # Change the name of the assigned object
    onebefore <- objeto_auxiliar[time_interval, on = .(Datetime >= start, Datetime
<= end),
                              by = .EACHI, .(
                                Datetime = i.Datetime, .N,
                                # Compute VeDBA values for second device
                                VeDBA.settable_device = if (.N < n_min) NA_real_ else
sum(sqrt(AccX ^ 2 + AccY ^ 2 + AccZ ^ 2)) / .N  # Formula para calcular actividad
en el intervalo de 57 segundos
                              )][,
                                 (2L:3L) := NULL][]

    # ADD COMMENT
    Comparison <- cbind(time_interval[,c("Datetime",
"VeDBA.reference_device")], onebefore)

    # Choosen statistical method will be computed
    if(correlation_method == "lm"){
      #print(Comparison)
      #readline(prompt="Press [enter] to continue")
      model <- lm(Comparison$VeDBA.reference_device ~
Comparison$VeDBA.settable_device)      # Hago una regresión lineal entre valores
calculados de actividad por el V13AP y el X16-mini
      resultado1_lm <- summary(lm(Comparison$VeDBA.reference_device ~
Comparison$VeDBA.settable_device))$r.squared # Extraigo R²
      # Extraemos el p-valor
      resultado2_lm <- summary(model)$coefficients[8]
      resto_variables <- data.frame("tiempo" = i, "r-square" = resultado1_lm, "p-
valor" = resultado2_lm)
```

```
          write.table(resto_variables, file = paste0(results_directory, "/", correlation_method, cases, ".txt"), col.names = FALSE, append = TRUE, row.names = F) # Creo archivo con R² y SE para distintos delays del X16 con respecto al V13AP
        }
        if (correlation_method %in% c("spearman", "pearson")){
          resultado_sp <- cor(Comparison$VeDBA.reference_device, Comparison$VeDBA.settable_device,  method = correlation_method, use = "complete.obs")
          resto_variables <- data.frame("tiempo" = i, "r-square" = resultado_sp)
          write.table(resto_variables, file = paste0(results_directory, "/", correlation_method, cases, ".txt"), col.names = FALSE, append = TRUE, row.names = F)
        }
        final_results <- rbind(resto_variables, final_results)
      }
      # The original DF is not changed to “data.table” format. To work with it, we must transform it again to “data”.
      reference_device <- as.data.frame(reference_device)
    }

    # We sum up the results with the mean value
    promedio <- final_results %>%
      # If we do not want to consider R^2 values with a p-value >0.05
      #filter(p.valor =< 0.05) %>%
      group_by(tiempo) %>%
      summarise_all(funs(mean,sd))
  }
}

### Plotting results ####
  ## Run this when using linear-model to measure correlation ####
if(correlation_method == "lm"){
  # Plot
```

```
  grafico <- ggplot(promedio, aes(x=tiempo, y=r.square_mean)) +
    geom_errorbar(aes(ymin=r.square_mean-r.square_sd,
ymax=r.square_mean+r.square_sd), width=.1) +
    geom_point() +
    geom_line() +
    labs(title= "",x= '\nTime delay',y="\n Mean R² +- sd") +
    theme(plot.title = element_text(hjust=0.5, face="bold",margin = margin(0,0,12,0)
),axis.title.y =element_text(margin = margin(t = 0, r = 12, b = 0, l = 0)),axis.title.x
=element_text(margin = margin(t = 12, r = 0, b = 0, l = 0))) +
    scale_x_continuous(breaks=round(seq(from = min(promedio$tiempo), to =
max(promedio$tiempo), by=abs(time_delay[2]-time_delay[1])),1))
  print(grafico)
}
  ## Run this when using Pearson's or Spearrman's to measure correlation
####
if(correlation_method %in% c("spearman", "pearson")){
  # Plot for "pearson" or "spearman"
  grafico <- ggplot(promedio, aes(x=tiempo, y=mean)) +
    geom_errorbar(aes(ymin=mean-sd, ymax=mean+sd), width=.1) +
    geom_point() +
    geom_line() +
    labs(title= "",x= '\nTime delay',y="\n Mean correlation strength +- sd") +
    theme(plot.title = element_text(hjust=0.5, face="bold",margin = margin(0,0,12,0)
),axis.title.y =element_text(margin = margin(t = 0, r = 12, b = 0, l = 0)),axis.title.x
=element_text(margin = margin(t = 12, r = 0, b = 0, l = 0))) +
    scale_x_continuous(breaks=round(seq(from = min(promedio$tiempo), to =
max(promedio$tiempo), by=abs(time_delay[2]-time_delay[1])),1))
  print(grafico)
}
```

Appendix B. R-code for temperature data-loggers synchronisation. The parts of the code underlined in blue indicate where the code needs to be configured to correctly run the synchronisation code, with small clarifications on these parts of the code in green notes.

```
### Global options ####
options("digits.secs" = 3)
set.seed(1)

### Libraries ####
library(dplyr)
library(lubridate)
library(data.table)
library(ggplot2)

### Comments ####

# Colum names for data of devices:
        # Date and time → Datetime
        # Temperature for the reference device → Tref
        # Temperature for the settable device → Tset


### Data load ####
  # Device 1 ####
reference_device <- readRDS("…")  # Directory where data for the reference device is located.

  # Device 2 ####
settable_device <- readRDS("...")  # Directory where data for the settable device is located.

### Results directory and object ####
```

```
results_directory <- "…"       # Directory where we want to save the files generated for the sync process.
final_results <- data.frame()

### Function declaration ####
data_domain <- function(tipo_analisis){
  #' Function to preprocess the original database depending the input data
  #'
  #' @description P
  #'
  #' @param tipo_analisis character: "acc", "temp"
  #' @usage data_domain("acc")

  if (tipo_analisis == "temp"){
    names(reference_device) <<- c("Datetime", "Tref")
    names(settable_device) <<- c("Datetime", "Tset")
    dataRaw <<- as.data.frame(reference_device[,1])
    names(dataRaw) <<- "Datetime"
    ### 'Datetime'  type ####
    reference_device$Datetime <<- as.POSIXct(reference_device$Datetime, format = "%Y-%m-%d %H:%M:%0S", tz = "UTC")
    settable_device$Datetime <<- as.POSIXct(settable_device$Datetime, format = "%Y-%m-%d %H:%M:%0S", tz = "UTC")

    # Results directory
    dir.create(file.path(paste0(results_directory, tipo_analisis)), showWarnings = TRUE)
  }
}
sync_temp <- function(data, originT, lowerW, upperW, delay){
  #' Function to create a vector column with new time activities interval in which VeDBA values must be calculated
  #'
  #' @description Calcultate aactivity intervals in which compute VeDBA values
```

```
  #'
  #' @param originT integer The time (in hours) that must be added to the first timestamp.
  #' @param lowerW integer The lower (in hours) time window value
  #' @param upperW integer The upper (in hours) time window value
  #' @param delay integer The delay. Activity duration window (in hours)
  #' @usage sync_temp(originT, lowerW, upperW, delay)
  #' @return A dataframe.
  #' @details The input must be four numeric values
  #'

  # Re-calculated first timestamp of the data, once we have added the originT.
  # This is perfom to ensure that time can be substracted in further steps when comparing interval windows before t0
  # data must be a dataframe
  first_value <- data[1, c("Datetime")]

  # Last timestamp of our object
  last_value <- data[nrow(data), c("Datetime")]

  # Updated timestamp value. The first value of "updated_value" is "first_value", then it is re-calculated
  updated_value <- first_value

  # A dataframe in which the mid-results are stored
  df <- data.frame(Date = as.POSIXct(character()),
                   Sampling_delay = integer(),
                   Initial_activity = as.POSIXct(character()),
                   Final_activity = as.POSIXct(character()))

  # Benchmark
  inicio <- Sys.time()

  # Loop that ends once the "updated_value" is after the "last_value"
```

```
  while(last_value >= updated_value){
    # A row with the results are computed.
    # Data frame structure must be used
    result <- as.data.frame(updated_value) %>%
      # We randomly select a value between the lower and upper window interval
      mutate(Sampling_delay = sample(lowerW:upperW, 1, replace = TRUE),
             Initial_activity = updated_value + hours(Sampling_delay),
             Final_activity = Initial_activity + hours(delay))

    # Result row is added to the declared df
    df <- rbind(df, result)

    # "updated_value" is updated
    updated_value <- result$Final_activity
  }

  # Benchmark
  fin <- Sys.time()
  # Execution duration
  print(fin-inicio)

  return(df)
}

### Setting parameters ####

sampling_frequency <- 1     # Number of records per unit of time (here is 'hour').
threshold <- 0.9        # Minimum proportion of samples per unit of time to consider that sampling window.
sampling_window_size <- 24   # Size of the sampling window (here 24 hours).
n_iterations <- c(1:15)       # Number of iterations per combination of configurable parameters.
time_delay <- seq(from = -72, to = 72, by = 4)     # TDR ("from" and "to") and TDRes ("by").
```

```
lower_sampling_delay <- 96  # Mimimum time distance between consecutive samples.
upper_sampling_delay <- 168  # Maximum time distance between consecutive samples.

### Statistical method to measure correlation strength ####
# Options: "spearman", "pearson" or "lm" (linear-model)

correlation_method <- "spearman"

approach <- "temp"

if (approach == "temp"){
  data_domain("temp")
  data_object <- dataRaw
  first_value <- data_object[(which(data_object$Datetime > data_object[1,] +
hours(max(time_delay)))), c("Datetime")][1]
  last_value <- data_object[(which(data_object$Datetime >
data_object[nrow(data_object),] -
hours(abs(min(time_delay))+sampling_window_size+1))), c("Datetime")][1]
  n_min <- sampling_frequency * sampling_window_size * threshold
  # Temperatura
  for(cases in n_iterations){
    print(paste0("Iteration number: ", cases))
    # We run the function
    time_interval_dev1 <- sync_temp(data = data_object, originT = max(time_delay),
lowerW = lower_sampling_delay, upperW = upper_sampling_delay, delay =
sampling_window_size)
    time_interval_dev1 <- time_interval_dev1[time_interval_dev1$Initial_activity >=
first_value & time_interval_dev1$Initial_activity <= last_value,]
    time_interval_dev2 <- time_interval_dev1

    # Only Initial_activity values are needed. We select and rename it to "Datetime"
    time_interval_dev1 <- time_interval_dev1 %>% select(Datetime = Initial_activity)
    time_interval_dev2 <- time_interval_dev2 %>% select(Datetime = Initial_activity)
```

```
  # Tibble are converted to Data_table structure
  # Here the delay must be specified again
  setDT(time_interval_dev1)[, Datetime := as.POSIXct(Datetime, format = fmt, tz =
"UTC")][,c("start", "end") := .(Datetime, Datetime + hours(sampling_window_size))]
  setDT(reference_device)[, Datetime := as.POSIXct(Datetime, format = fmt, tz =
"UTC")]

  # ADD COMMENT
  time_interval_dev1 <- reference_device[time_interval_dev1, on = .(Datetime >=
start, Datetime <= end),
                      by = .EACHI, .(
                       Datetime = i.Datetime, .N,
                       T.reference_device = if (.N < n_min) NA_real_ else Tref
                      )][,(2L:3L) := NULL][]

  time_interval_dev1 <- time_interval_dev1[time_interval_dev1$N>=n_min,]

  # In case all N value es lower than the minimun N, we stop the execution
  if(all(time_interval_dev1$N<n_min)){
   print("N is lower than n_min")
   break
  }
  else{
   # Remove observations in which less than n_min actity points are used

   # ADD COMMENT
   for (i in time_delay){
    objeto_auxiliar <- settable_device
    objeto_auxiliar$Datetime <- objeto_auxiliar$Datetime + hours(i) # Comprobar
que sale bien

    setDT(time_interval_dev2)[, Datetime := as.POSIXct(Datetime, format = fmt, tz
= "UTC")][,c("start", "end") := .(Datetime, Datetime +
hours(sampling_window_size))]  # "DetectionsHAleAcc" contiene los tiempos de
```

```
toma de actividad para los V13AP. Calcula actividad para 57 segundos. También contiene el valor de actividad para esos 57 segundos del V13AP
    setDT(objeto_auxiliar)[, Datetime := as.POSIXct(Datetime, format = fmt, tz = "UTC")]   # "objeto_auxiliar" son los datos del X16-mini con un "delay" determinado (que va desde -180 a +180)

    # Change the name of the assigned object
    onebefore <- objeto_auxiliar[time_interval_dev2, on = .(Datetime >= start, Datetime <= end),
                             by = .EACHI, .(
                               Datetime = i.Datetime, .N,
                               # Compute VeDBA values for second device
                               T.settable_device = if (.N < n_min) NA_real_ else Tset
                             )][,
                                (2L:3L) := NULL][]
    onebefore <- onebefore[onebefore$N>=n_min,]

    # ADD COMMENT
    Comparison <- cbind(time_interval_dev1[,c("Datetime", "T.reference_device")], onebefore[,c("T.settable_device")])

    # Choosen statistical method will be computed
    if(correlation_method == "lm"){
      #print(Comparison)
      #readline(prompt="Press [enter] to continue")
      model <- lm(Comparison$T.reference_device ~ Comparison$T.settable_device)         # Hago una regresión lineal entre valores calculados de actividad por el V13AP y el X16-mini
      resultado1_lm <- summary(lm(Comparison$T.reference_device ~ Comparison$T.settable_device))$r.squared # Extraigo R²
      # Extraemos el p-valor
      resultado2_lm <- summary(model)$coefficients[8]
      resto_variables <- data.frame("tiempo" = i, "r-square" = resultado1_lm, "p-valor" = resultado2_lm)
```

```
          write.table(resto_variables, file = paste0(results_directory, "/", correlation_method, cases, ".txt"), col.names = FALSE, append = TRUE, row.names = F) # Creo archivo con R² y SE para distintos delays del X16 con respecto al V13AP
        }
        if (correlation_method %in% c("spearman", "pearson")){
          resultado_sp <- cor(Comparison$T.reference_device, Comparison$T.settable_device, method = correlation_method, use = "complete.obs")
          resto_variables <- data.frame("tiempo" = i, "r-square" = resultado_sp)
          write.table(resto_variables, file = paste0(results_directory, "/", correlation_method, cases, ".txt"), col.names = FALSE, append = TRUE, row.names = F)
        }
        final_results <- rbind(resto_variables, final_results)
      }
      # The original DF is not changed to “data.table” format. To work with it, we must transform it again to “data”.
      data_object <- as.data.frame(data_object)
    }

    # We sum up the results with the mean value
    promedio <- final_results %>%
      # If we do not want to consider R^2 values with a p-value >0.05
      #filter(p.valor =< 0.05) %>%
      group_by(tiempo) %>%
      summarise_all(funs(mean, sd))
  }
}

### Plotting results ####
  ## Run this when using linear-model to measure correlation ####
if(correlation_method == "lm"){
  # Plot
```

```
  grafico <- ggplot(promedio, aes(x=tiempo, y=r.square_mean)) +
    geom_errorbar(aes(ymin=r.square_mean-r.square_sd,
ymax=r.square_mean+r.square_sd), width=.1) +
    geom_point() +
    geom_line() +
    labs(title= "",x= '\nTime delay',y="\n Mean R² +- sd") +
    theme(plot.title = element_text(hjust=0.5, face="bold",margin = margin(0,0,12,0)
),axis.title.y =element_text(margin = margin(t = 0, r = 12, b = 0, l = 0)),axis.title.x
=element_text(margin = margin(t = 12, r = 0, b = 0, l = 0))) +
    scale_x_continuous(breaks=round(seq(from = min(promedio$tiempo), to =
max(promedio$tiempo), by=abs(time_delay[2]-time_delay[1])),1))
  print(grafico)
}
  ## Run this when using Pearson's or Spearrman's to measure correlation
####
if(correlation_method %in% c("spearman", "pearson")){
  # Plot for "pearson" or "spearman"
  grafico <- ggplot(promedio, aes(x=tiempo, y=mean)) +
    geom_errorbar(aes(ymin=mean-sd, ymax=mean+sd), width=.1) +
    geom_point() +
    geom_line() +
    labs(title= "",x= '\nTime delay',y="\n Mean correlation strength +- sd") +
    theme(plot.title = element_text(hjust=0.5, face="bold",margin = margin(0,0,12,0)
),axis.title.y =element_text(margin = margin(t = 0, r = 12, b = 0, l = 0)),axis.title.x
=element_text(margin = margin(t = 12, r = 0, b = 0, l = 0))) +
    scale_x_continuous(breaks=round(seq(from = min(promedio$tiempo), to =
max(promedio$tiempo), by=abs(time_delay[2]-time_delay[1])),1))
  print(grafico)
}
```

Appendix C. Effect of the sampling-window size as a function of the formula used to estimate activity (RMS vs aVeDBA).

In acoustic telemetry, accelerometers typically calculate activity using an algorithm called RMS (Eq. 1), which is assumed to be analogous to VeDBA (Eq. 2) (Cooke et al. 2016), whose use is common in disciplines where raw acceleration data from accelerometers are available (Gleiss et al. 2010, Qasem et al. 2012, Lyons et al. 2013, Wright et al. 2014, Thiem et al. 2015). The formulas for RMS and VeDBA are:

$$RMS = \frac{\sqrt{\sum_{i=1}^{n}\left(X_{dyn}^{2} + Y_{dyn}^{2} + Z_{dyn}^{2}\right)}}{n} (Eq. 1)$$

$$VeDBA = \sqrt{\left(X_{dyn}^{2} + Y_{dyn}^{2} + Z_{dyn}^{2}\right)} (Eq. 2)$$

where X_{dyn}, Y_{dyn} and Z_{dyn} represent the dynamic acceleration (i.e., remainig acceleration after removing the static one) (m s^{-2}) in the X, Y and Z axes, respectively; and *n* the number of acceleration measurements performed by the accelerometer, which results from multiplying the sampling-window size by the recording frequency.

As it is shown, VeDBA applies a square root over the sum of squared dynamic accelerations, but it does not incorporate the term '*n*', as it is a statistic calculated on each accelerometer acceleration measurement. RMS, instead, applies the square root after the sum of the squared dynamic accelerations recorded by the accelerometer for a given sampling-window and divides it by the number of measurements (i.e., RMS is an average). This might convert the RMS formula very sensitive to the SWS since the squared dynamic accelerations value over which it is performed the square root changes depending on the SWS.

Therefore, we designed a new formula called 'average VeDBA' or 'aVeDBA', whose equation is

$$aVeDBA = \frac{\sum_{i=1}^{n}\sqrt{\left(X_{dyn}^2+Y_{dyn}^2+Z_{dyn}^2\right)}}{n}\,(Eq.3);$$

which is analogous but not equal to RMS since

$$\frac{\sqrt{Acc_{t1} + Acc_{t2}+\ldots+Acc_{tn}}}{n} \neq \frac{\sqrt{Acc_{t1}} + \sqrt{Acc_{t2}}+\ldots+\sqrt{Acc_{tn}}}{n}\,(Eq.4);$$

where *Acc* represent the sum of square dynamic accelerations (i.e., Acc = X^2_{dyn} + Y^2_{dyn} + Z^2_{dyn}) for a given time (*t*), *n* the number of acceleration measurements, the left expression shows the RMS formula and the right one the aVeDBA formula. As it is seen, in the aVeDBA case, the square root is applied to each sum of square dynamic accelerations on the three axes corresponding to a single acceleration measurement by the accelerometer and then added together. Thus, aVeDBA is likely less sensitive to the SWS compared to RMS since the possible effect associated to the addition of squared dinamic accelerations before applying the square root is removed.

To assess the sensitivity of RMS and aVeDBA to the sampling-window size (SWS), we explored how the overall mean activity (henceforth OMA) changed as a function of the formula (RMS vs aVeDBA), the SWS (8 levels: 15 s, 25 s, 35 s, 45 s, 55 s, 65 s, 75 s, 85 s) and the type of fish behaviour. To do so, on the one hand, we simulated two distinct fish behaviours that we called "regular" and "irregular". This was performed placing an X16-mini in a physical object with a similar orientation to that of the fishes and recording acceleration at a frequency of 12 Hz (the same than in wild dusky groupers used in this thesis), simulating different movements of fish. In particular, we recreated four movements (burst, intense swimming, soft swimming and resting) for several seconds each one (Fig. 1). The regular behaviour consisted of the sequential repetition of the "soft swimming" movement for 5 hours. The irregular one was a random mix of these four movements for 5 hours, yet, some movements predominated over others (resting > soft swimming > intense swimming > burst) to make it similar to the assumed behaviour of the dusky groupers. We assume the acceleration signals are likely far from being identical to the ones produced by a dusky grouper. However, we believe this is not a constraint since our goal is just to compare RMS and aVeDBA performances in two extreme scenarios. On the other hand, we used real fish behaviours through raw accelerations from the six tri-axial accelerometers we recovered from six dusky groupers. It is important to note that the difference between simulated and real fish data is that we did not know the intensity of the movement in the latter case.

As for the results concerning simulated data, preliminary results evidence that the SWS effect on OMA varies among the behaviours 'Irregular' and 'Regular' depending on the formula (RMS vs aVeDBA): while no differences in OMA were found among SWS when using aVeDBA, OMA varies among SWS for RMS for the 'Irregular' behaviour but not for the 'Regular' one (Fig. 2). The results about the SWS effect depending on the formula when using real dusky grouper activity data show that OMA was affected by the SWS for RMS but not for aVeDBA (Fig. 2).

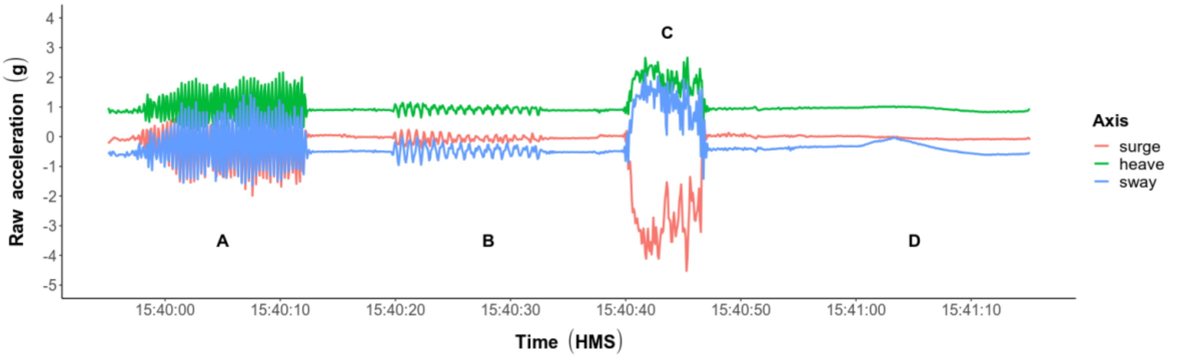

Figure 1. Plot showing raw acceleration in g force (1g= 9.8 m s^{-2}) for the X (surge), Y (heave) and Z (sway) axis at 12 Hz overtime for the simulated behaviours "intense swimming" (A), "soft swimming" (B), "burst" (C) and "resting" (D).

Hence, it was only for the aVeDBA formula for which OMA showed to be constant among SWS for the different data sources (either simulated or real) (Fig. 2). This suggests aVeDBA as a better activity estimator than RMS since it is more robust to SWS changes or different type of behaviours. RMS formula was robust to SWS changes only for regular behaviours, which suggest that the 'additive effect' associated with the sum of square dynamic accelerations before performing the root mean square is not important when activities are constant over time, something rare in the real world. Although RMS is a minor problem for independent studies (i.e., comparisons can be made among individuals that had the same acceleration transmitter settings), they may represent a significant problem when comparing activity levels among individuals/species from different studies since they will likely use different SWS.

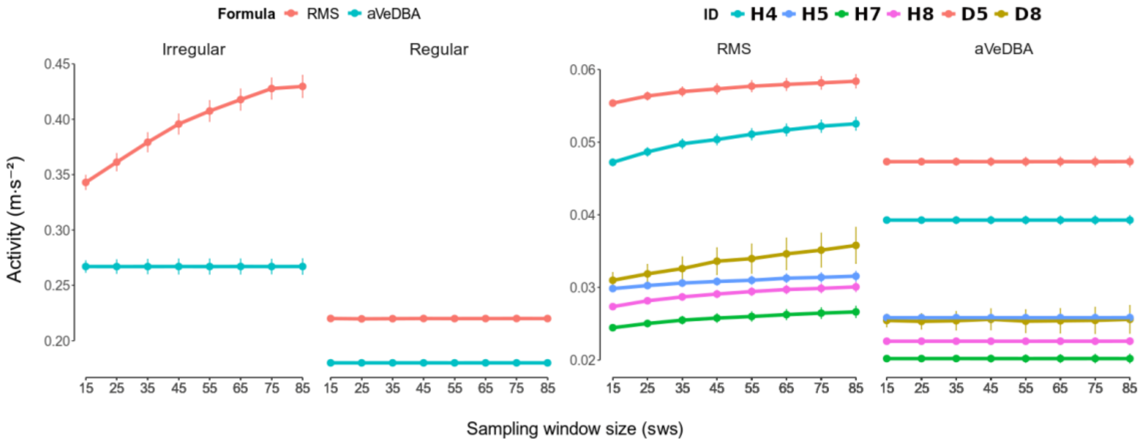

Figure 2. Plots showing the overall mean activity (OMA) response (y-axis) to the sampling-window-size and formula for simulated behaviours (left) and to the sampling-window-size and formula per individual for real data (right).

The use of acceleration transmitters is expected to increase considerably in the coming years (Cooke et al. 2016). Thus, it is important to use an activity formula robust to the use of different SWS. That is, a study using a SWS of 16 s (e.g., Udyawer et al. 2017) and another one using a SWS of 45 s (e.g., Runde et al. 2020) will be able to compare activity values with more confidence if they use aVeDBA than if they use RMS. The reason why RMS is more affected by the type of SWS and behaviour is likely related to the equation. As we mentioned previously, the RMS equation performs the square root once the sum of the squared dynamic accelerations of the three-axis over the entire sampling-window has been done, which means that depending on the SWS, the square root will be calculated over a larger o shorter number of dynamic accelerations. This is likely behind the results found. Therefore, and due to the simplicity of changing the type of formula used by the manufacturers, we advise changing the RMS formula to aVeDBA as this is more independent than RMS to the SWS and the type of animal behaviour.

Appendix D. Transformation from continuous to discrete activity values with the V13AP.

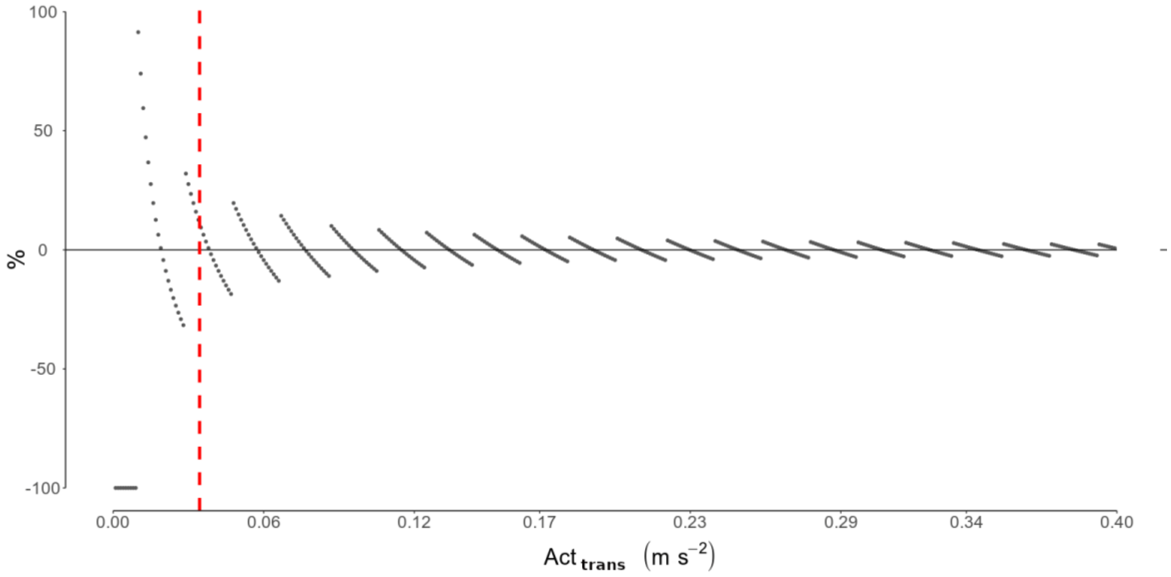

Illustration of the effect of the transformation of the original activity values recorded by the acceleration transmitter ‘V13AP’. Specifically, it represents the percentage of change (Y-axis) between the original (X-axis) and the final activity value obtained after downloading data from receivers. Thus, a positive 100% change means that the final activity value obtained was twice the original value. The minimum activity measured with the acceleration biologger in *E. marginatus* is indicated with a vertical dashed line. Note we did zoom to low activity values (<0.4 m s^{-2}), where this conversion issue becomes relevant.