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DO FISH SLEEP?

Sharpening fishy sleep criteria



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Abstract

Sleep research revealed the presence of sleep in a multitude of species; because it is such a widespread activity it leads to believe that sleep is a necessary process. Mammalian sleep research provided electrophysiological and behavioral sleep criteria; perhaps these criteria are sufficient to identify sleep in non-mammals as well. Sleep research has mainly focused on mammalian sleep and kept sleep research in other species in the background. Sleep studies have been done on reptiles and fish, but to far less extent than mammals. Because fish are ancient species, if sleep were found in them, it could perhaps explain the continuous presence of sleep within the animal kingdom.

Up to now electrophysiological sleep criteria (e.g. EEG and EMG measurements) have not been done in fish. It is not impossible to measure EEG in fish, it is however very difficult to do so. It is for that reason that many studies relied on behavioral sleep criteria (provided by the intense research done on mammalian sleep). It could be that these mammalian behavioral criteria are insufficient for sleep determination in fish. What is apparent, is the seemingly lack of structure within the fish sleep studies. Most researchers only used a few mammalian sleep criteria and added other criteria, while few did use all available sleep criteria to fully establish the presence of sleep or not.

Overall, fish sleep studies accept the presence of sleep in fish, even when there is little to no data available to reinforce their conclusion. If fish, an ancient line of the animal evolution, sleep then that would have great implications on the evolution of sleep. This could even affect current thinking on mammalian sleep. Why are we focusing on more recent evolved mammals and their sleep, when there are ancient species who might show more basic principles of sleep?

So far, the presence of sleep in fish is plausible, but much more data has to be collected in order to be certain of it. So far, fish are non-sleepers, until proven sleeping.

Keywords: *Sleep, Fish, Mammalian sleep criteria, Electrophysiological sleep criteria, EEG, EMG, Behavioral sleep criteria, review.*

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INTRODUCTION

Sleep has been studied extensively in mammals, and is now continuing its step to other animals as well. From what we have learned from mammalian sleep studies is that it is highly diverse. EEG sleep patterns found in a certain species do not necessarily resemble to EEG patterns in other sleeping species. Thus the use of EEG might become of import for official and final determination of sleep, after all behavioral sleep criteria have been satisfied (*Allada & Siegel 2008*).

If we are able to understand what drives an animal to sleep, might we then be able to answer why we sleep? According to *Kavanau 2006*, sleep is “the proverbial riddle wrapped in a mystery inside an enigma”. We need firm base rules to eliminate any ‘quiescent’ behavior that might resemble sleep. But what are these rules? And how do we use them to successfully identify sleep?

Current hypothesis identify sleep as a restorative function, necessary for the animal to commit, to ensure full brain capacity during wakefulness.

If in fact sleep is necessary, then disturbing/preventing the animal from sleeping would cause it to increase the duration or depth of its next sleeping bout (*Rial et al. 2007,2010, Allada & Siegel 2008, Siegel 2008, Duboué et al 2011*). This process is referred to as sleep rebound.

Thus far, studies have focused on sleep and wakefulness in mammals, more so than for instance fishes or reptiles. Fish sleep has become of increasing interest over the years in sleep studies, and whether the recognized mammalian sleep criteria apply to fish as well. But there is a long way before full comprehension of sleep occurs to us. Even in the extensively studied mammalian species, we still have much to learn.

Mammalian studies proposed that sleep duration might be linked to the possibility of being able to sleep safe. This would mean that fish swimming in schools would be able to sleep for longer periods of time, because they have group protection. But then, what of the diurnal active schools that break up during the night? They abandon group protection during the night, when they supposedly sleep, thus when they are most vulnerable (*Kavanau 1998*).

Fish lifestyles, like mammalian lifestyles, are extremely diverse. Some fish are pelagic deep diving and active swimmers, others roam the sea floor passively passing time, while others stay within reach of the coral-reef or other protective areas (*Grutter et al. 2010*). Even with very apparent lifestyle differences in mammalian species, studies agree that they all show signs of sleeping. So, perhaps, to some extent, the fish are sleeping as well?

Fish are ancient species and if sleep is present in these ‘dinosaurs’ then that might shed light on later evolved animals. Why haven’t studies primarily focused on the presence of sleep in fish? But most importantly, how much do we actually know about sleep in fish?

If in fact, fish do sleep, it becomes very intriguing for future studies on higher level animals.

First, we need to discover whether fish sleep at all. Resulting in the research question I wish to answer, do fish sleep?

DO FISH SLEEP?

Mammalian sleep studies form the basis for sleep recognition/determination in fishes. Studies on mammalian sleep have provided sufficient information for sleep identification, but are they sufficient enough to define sleep in fishes as well? And if they

are sufficient, according to these criteria, are the fish actually sleeping?

SLEEP CRITERIA

Mammalian sleep studies revealed certain criteria necessary to identify sleep (Kavanau 2004, 2006, Aparicio et al. 2007, Rattenborg et al. 2007, Allada & Siegel 2008, Cirelli & Tononi 2008, Siegel 2008, Lima et al. 2005). These criteria can belong to one of two categories, electrophysiological or behavioral.

The first category, **electrophysiological sleep criteria**, consists of EEG and EMG measurements (Electroencephalogram and Electromyography resp.). This category is frequently used in mammalian sleep studies. EEG uses brain wave measurements and it reveals peaks and patterns of those brain waves (See Fig. 1) (Bryant et al. 2004, Siegel 2008, Lesku et al. 2009).

Non-REM has several stages of consciousness, higher amplitude EEG coincides with a lower level of consciousness. The highest amplitudes are known as slow wave NREM sleep. During stages 3 and 4 the subject has the lowest level of consciousness and is most likely combined with an increased arousal

threshold (Allada & Siegel 2008, Siegel 2008, Lesku et al. 2009).

REM sleep has only one stage, and amplitude size resembles that of the first stage of NREM sleep (Fig. 1).

REM sleep varies significantly from Non-REM sleep; REM does have increased amplitude when compared to conscious levels. However, these amplitudes are much smaller compared to NREM sleep. In this state the animal is asleep and more alert than that of a slow-wave NREM sleeper. This coincides with a decrease in arousal threshold. (Bryant et al. 2004)

EEG is the most sensitive and accurate of present sleep methods to distinguish sleep from wakefulness and allows for a further distinction between different sleep stages, particularly NREM and REM sleep. However fish do not have that mammalian neo-cortex and do not show similar patterns found in mammals when sleep kicks in (McGrouther 2003). In the case of fish sleep, EEG might not prove to be as useful as in mammalian sleep studies. Hence the necessity to increase the use of mammalian sleeps criteria for identification.

EMG measures the electrical output of a certain muscle or a group of muscles. An

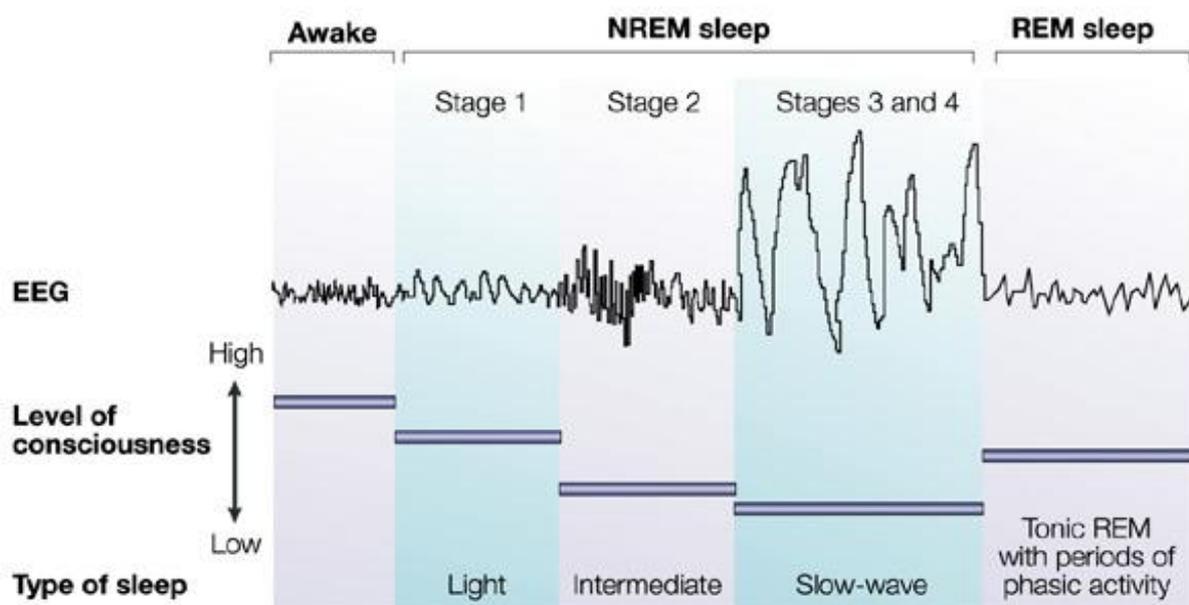


Figure 1. Stages of sleep, combined with EEG measurements. When the level of consciousness decreases (an increase in sleeping depth), EEG waves increase in size. Therefore EEG measurements would be most useful for sleep-rebound determination. - Bryant et al. 2004

increase in muscle activity indicates the muscle is actively used and usually indicates wakefulness (Dewar *et al.* 1999).

There is a close correlation between EEG amplitudes and behavior in most of the mammals that have been studied so far (Tobler 1995). Thus when behavioral observations are excluded (not visible) then EEG measurements have become a method of choice for sleep investigation (Tobler 1995).

Hobson (1967) provided a detailed study of activity and responsiveness in the bullfrog (*Rana catesbeiana*). The frog has diurnal activity however showed to be more responsive during periods of inactivity than when they were active. Hobson suggested that the frogs 'only survived because they rest without loss of vigilance'. They concluded that the bullfrog didn't sleep.

A second study regarding the same observer, yet this time the tree frog (*Hyla septentrionalis*) as test-subject, concluded that the species do sleep (Hobson *et al.* 1968). Both species show no evidence of REM presence and both their EEGs show a maximum voltage in their forebrains while in an alert and active state. When they are resting they both show minimum voltage in their forebrain EEGs. This is opposite of what any other mammalian sleep study has so far observed (Siegel 2008). And proves that rest and sleep states are not equal amongst the species, sleep must be defined behaviorally as well.

Measuring EEG and EMG is however a complex task to perform in fishes. Because they live in water, micro-electrical signals are easily dispersed and lost. So far, no attempt has been made to measure EEG in fish. EMG measurements, on the other hand, have been done in a few free-swimming fishes by a study from Dewar *et al.* 1999. The emphasis of the study was however not on

sleep, but on the behavior and physiology of a free-swimming fish. Nevertheless, they prove that it is possible to measure EMG in fish, something that could be incorporated in future sleep studies.

The second group, **behavioral criteria**, consists of many 'sub' – observations/criteria. Behavioral criteria might not have the same accuracy as EEG or EMG measurements but they can provide relevant information on overall sleeping behavior and for some species, EEG and EMG measurements are too complicated to perform. Both of the processes depend on small electrical pulses and currents. For them, behavioral sleep criteria offer the only possibility to study sleep.

However that means that even if the animal in question shows all the behavioral cues, it might be impossible to conclude that the animal is sleeping. Do we need further addition of EEG and EMG measurements to validate that?



Figure 2. Sperm whales (*Physeter macrocephalus*) sleeping posture vs. seals. The sperm whales show a very distinctive sleep posture, which does not resemble their wakeful 'posture'. The seal also shows clear sleep-posture and can easily be recognized from an active state.

Immobility, the animal is in a visible inactive state (Rial *et al.* 2007, Yokogawa *et al.* 2007, Lesku *et al.* 2009). Counter intuitively, this is not as straight forward as it may seem. For instance, dolphins rely on uni-hemispheric sleep, which allows them to swim continuously while one half of the brain is 'resting' (Siegel *et al.* 2008). Regarding the immobility criterion, it would

appear that the dolphin is continuously awake. And it is not the only one showing continuous activity, as will be described in fishes later on. However their swimming was discrete and low key, thus very different from wakeful swimming (Allada & Siegel 2008, v.d. Klij unpubl.)

McGrouther (2003) made an interesting statement regarding sleep in fishes – “Many fishes seem not to sleep. Pelagic species such as tunas and some sharks never stop swimming. One idea is that, during sleep, sensory information (predominantly visual) gathered during the day is processed to form memories. Fishes that swim constantly in blue oceanic waters receive little ‘unusual’ visual input and require less ‘memory-processing time’ and thus need no sleep.” Note that McGrouther disregards any other type of sensory input as a possible onset of sleep, which I will elaborate on later.

By being continuously active the entire day/night they ‘violate’ one of the behavioral criteria regarding sleep, but is that enough to conclude that the animal is incapable of sleep? I would disagree, for there are many non-pelagic species of fish which remain active throughout the night, yet seem to show other behavioral signs for sleep (Goldshmid et al. 2004).

Typical posture, Shapiro & Hepburn 1975 & Hendricks et al. 2000, argued that this behavioral criterion is species specific. But in general, a recurring posture, which is quiescent in nature and different from that of the animal in activity, is one of the behavioral signs of sleep. (Fig. 2) But would the quiescent night-swimming of a coral reef fish species count as a typical posture? There seems to be little information on the specifics of this criterion.

Typical place/nest, the animal retreats to a safe place, for instance, within the coral reef (Campbell & Tobler 1984) or in a self-built mucous cocoon (e.g. the parrotfish) (Grutter

et al. 2010, Fig. 3). In general, the animal moves from rather dangerous open water (where it is active) to a ‘safer’ and smaller (e.g. coral reef) area. Once the animal is safe, it refrains from activity, settling into a cocoon or set itself to ‘quiescent night swimming’ (Kavanau 2004, Siegel et al. 2008).



Figure 3. Parrotfish (*Scaridae* sp.) in a mucous ‘cocoon’ by own creation. In which it supposedly sleeps during the night (Grutter et al. 2010).

Typical time of day/circadian rhythm, this is related to the behavioral criteria of a typical ‘resting’ place or nest. There are three types of activity, diurnal, nocturnal or crepuscular. Most mammals are diurnal and show signs of immobility at set periods of the day (Siegel 2008, Hur et al. 2012). However, studies testing the species circadian rhythm found that light exposure in some species temporarily deleted their entire daily rhythm. In case of the zebrafish, a sudden light exposure kept the fish active for a maximum of eight days before it returned to its pre-stimulated rhythm (Duboué et al. 2011, Hur et al. 2012). Their continuous wakefulness is not considered ‘normal’ zebrafish behavior, thus light ‘over’-exposure should be avoided during experiments. How light affects the zebrafish’ daily rhythm is yet to be determined.

Elevated arousal threshold, sleeping mammals have higher arousal thresholds (Yokogawa et al. 2007, Allada & Siegel 2008, Rial et al. 2010, Duboué et al. 2011). They

seem less aware of their surroundings than they do during wakefulness. Either the animal responds less quickly to the external stimulus or it needs a stronger stimulus to create a response. When there is an increase in response time or an increased external stimulus necessary to wake the animal, said animal shows a behavioral criterion of sleep, an increase in arousal threshold. (Allada & Siegel 2008, Duboué et al. 2011). External stimuli can either be sound, light or even vibrations.

Quick reversibility, this criterion goes hand in hand with arousal threshold. If the 'sleep-state' can't be reversed, then it is likely that something else is causing this sleep-like state. For instance, the animal had all the behavioral criteria for sleep, however does not arouse when stimulated, one might argue that the animal is in fact in a coma, hibernation or dead (Yokogawa et al. 2007, Zimmerman et al. 2008).

Homeostatic regulation, defines the need for sleep to occur, hence a regulation of sleep. If the animal were deprived of sleep, would it increase its following sleep duration and/or depth? An increase in either of these is categorized as sleep rebound (Yokogawa et al. 2007, Allada & Siegel 2008, Siegel 2008, Duboué et al. 2011). Whether sleep rebound is either measurable as an increase of sleep time or sleep depth, remains a topic of discussion. Most researchers argue an increase in sleep duration to be the only true measure for sleep rebound, just a few disagree and suggest that sleep depth is more important for rebound determination.

In order to settle this conflict we would need to test the subjects for arousal threshold levels; if they are increased during 'rebound sleep' (after a sleep deprivation experiment) it strongly suggests that the animals take on a deeper form of sleep.

Sleep rebound is proposed to be a very important criterion for sleep recognition. Sleep researchers argue that without the presence of sleep rebound in an animal, the animal has no need to sleep at all, hence sleep would have no function in the individual (Rattenborg et al. 2007).

These criteria are the behavioral sleep criteria necessary to identify sleep. But how have they been used in the field? What types of sleep studies have so far been done, and which criteria did these studies use?

FISH SLEEP STUDIES

Who focused on sleep in fishes so far? And how have they determined/classified sleep in these fishes? What behavioral criteria do they use? What were their results? I wish to answer these questions through analysis of current knowledge regarding sleep research.

In 2011 Duboué et al. studied sleep in 4 species of surface-/cave-fish closely related species of fish (*Atyanax mexicanus*), hybrid cavefish (from 3 caves; Pachon, Tinaja, Molino) and the surface fish (Fig. 4). The cavefish is blind and lives in dark cave areas, the surface fish are fully sighted and live in open areas.

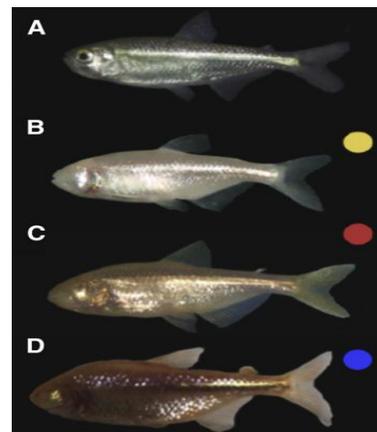


Figure 4. *Atyanax mexicanus*, surface fish (A) & hybrid cavefish: Molino (B), Pachon (C) Tinaja (D) – (Duboué et al. 2011)

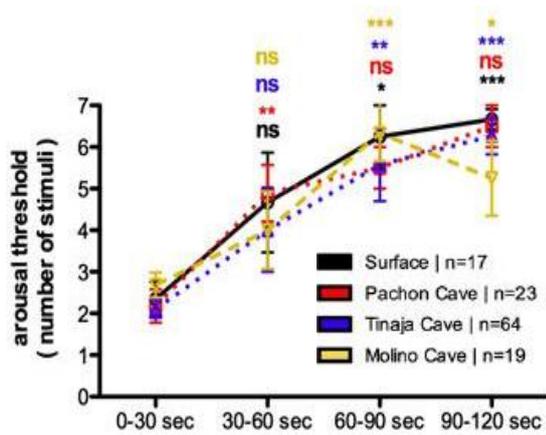


Figure 5. Arousal threshold measurement as number of stimuli (on the y-axis) needed to evoke a response. The x-axis shows the different levels of resting time prior to application of external stimulus. With increasing resting time prior to stimulation, the amount of stimuli necessary to evoke response increased as well. Colors indicate the different species of surface and cave-fish. (Duboué et al. 2011)

Duboué et al. relied only behavioral sleep criteria for sleep recognition. The four species of fish were studied during their inactive state (**Immobility**, the study does not mention posture or certain place/nest of the fish). They quantified velocities to be able to differentiate between true sleep and reduced activity. They found active waking velocities of the cavefish (Pachon & Tinaja) to be significantly higher than that of the surface fish. The Molino Cavefish, however, were not significantly different from the surface fish. They also noted differences in average sleep time, and amount of sleeping bouts. Average night sleeping bout duration was highly significant between the cave and surface fish (Table 1.). An average night sleeping bout of the surface fish lasted 42 minutes, while that of the Molino cave-fish species lasted 1.2 minutes.

	Surface	Pachon	Tinaja	Molino
'Sleep' time (min)	819.3±68.6	242.7±76.3	107.4±13.7	136.88±38.5
'Sleep' bout (#) Day	47.71±6.3	12.61±5.5	24.6±2.7	30.94±7.3
'Sleep' bout (#) Night	31.1±7.3	44.71±8.9	28.19±2.6	42.55±10.0
Average bout duration (min) Day	5.49±1.2	2.07±1.3	1.52±0.1	1.2±0.3
Average bout duration (min) Night	42.41±13.1	3.85±1.6	2.2±0.2	1.2±0.3

Table 1. Results from sleep research Duboué et al. 2011. Sleep time, sleep bout number and average time of sleep bout was calculated. Surface fish have the longest sleep time, which is most prominent at night. The Tinaja cavefish species has the shortest sleep time, while the Molino cavefish has the shortest average sleeping bout duration during both day and night.

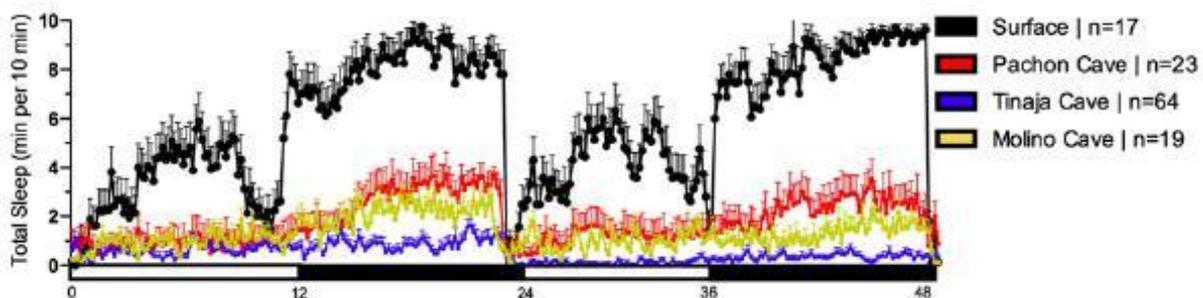


Figure 6. Total sleep duration in minutes per 10 minutes measured over a 48 hour period of time. Graph reveals the Surface fish to be highly diurnal as they sleep most during the night. The three cave-fish species; Pachon, Tinaja & Molino have a highly different sleeping pattern compared to the Surface -fish, and lack a clear diurnal or nocturnal pattern. These three cave fish species evolved independently from each other. The Cave-fish species show strongly reduced sleep duration compared to Surface-fish, which results from a convergent evolution. (Duboué et al. 2011).

The fish were externally stimulated, by using six mechanical stimuli (gentle standardized tapping every 2 seconds) per level of inactivity prior to stimulation (grouped the levels as; 0-30 seconds, 30-60, 60-90 & 90-120 seconds). Thus the second group was inactive for 30-60 seconds prior to starting the six mechanical stimuli.

If the first stimulus registered a response, the experiment was stopped and the individual was marked to respond at arousal threshold level 1 (Fig. 5).

Between each of these time levels (seconds of inactivity prior to stimulation) the experiment was halted for 20 minutes, allowing the fish to 'recover'.

Duboué et al. 2011, calculated arousal threshold as mean number of taps needed to elicit a startle response. This was significantly higher in individuals who were inactive for over 60 seconds prior to stimulus application. Only the Pachon Cave fish population shows a significant elevated arousal threshold at 30-60 sec prior to stimulation.

The longer the observer waited prior to stimulation correlated with an increase in arousal threshold (Fig. 5). They found that their subjects, after an inactivity of sixty seconds or longer had a significantly increased **arousal threshold**.

After establishing the presence of sleep, *Duboué et al* compared sleep of the surface-/cave- fish with each other.

They concluded an increase in response time as time of inactivity of the fish increased. This increase in response time was most apparent at the sixty second mark, thus they used that mark as a determination for sleep. If the surface-/cave- fish were inactive for sixty seconds or more, they are asleep.

Duboué et al. also used another criterion in order to determine whether this observed increase in arousal threshold was in fact sleep. Namely, the need for **sleep rebound**.

During their study they deprived the fish of sleep for twelve hours, by subjecting their fish to continuous vibration (4mm amplitude, 1 Hz) either during subjective day or night. After the 'sleep deprivation'-setup they found an increase in sleep duration afterwards. They concluded the fish's need for sleep rebound and that sleep was part of their homeostatic regulation.

In this case sleep rebound was proved by an increase in sleep duration; they ignored the other method for sleep rebound, namely increase in sleep depth. For that to be established, one would need to perform EEG measurements, or check whether they also show a further increase in arousal threshold. We thereby assume that a deeper sleep would need a stronger external stimulus to wake the subject (*Cirelli & Tononi 2008*).

Duboué et al. established a decrease in sleep duration in cavefish species compared to the surface fish (Fig. 6, Table 1.). They proposed that because the cavefish are blind and forage in dark areas, they have less incentive to sleep. Their brains no longer need the extra processing time, because their largest input (their eyes) do not function.

However, not all memories depend on sight, and for the cavefish to catch their prey successfully, they would need highly effective location skills. It seems that these skills also require the fish to rest, even though for a decreased period of time (as opposed to the sighted surface-fish).

ZEBRAFISH

The study of *Yokogawa et al. 2007* defined the sleep state in adult zebrafish (*Danio rerio*, Fig. 7) as: a **reversible** period of **immobility**, a period of an increased **arousal threshold** and **place preference** (at the bottom or top of the tank). They acknowledged the presence of **sleep rebound**, after longer periods of electrical stimulation deprived them of sleep. They

defined sleep rebound as an increase in sleep duration.



Figure 7. Zebrafish (*Danio rerio*) used as sleep study subject for numerous studies throughout the years. (Source: The Zebrafish model Organism Database, ZFIN)

In order to define sleep in fully formed adult zebrafish, they used infrared video-tracking (Adult fish sleep recording system, AFSRS *Zhadanova et al. 2001, Ziv et al. 2007*) of fish under both light and dark conditions (mimicking day and nighttime).

Studies revealed the zebrafish to be significantly more active during the day than during the night. They also observed brief periods of inactivity during the day and these moments of ‘immobility’ were often associated with a ‘drooping caudal fin’, depicting a certain behavioral **posture** of the fish (when supposedly resting). These moments of ‘immobility’ also occurred during both day and night times. In both cases the inactive ‘tail drooping’ state was easily reversed with external stimuli. They applied voltage stimuli (ranging from 0 – 2 volts) in random order, every thirty minutes throughout the entire day and night. The fish that were in an active state before being stimulated were observed to respond (from drooping caudal fin to erect fin, and increased movement) to very low voltage stimuli when compared to the fish in inactive state before stimulation. At higher voltages, however, all fish responded and regained wakefulness, regardless of their state prior to the stimulation.

Yokogawa et al. 2007 thus established that when periods of immobility increased, the arousal threshold increased as well. But that it was a finite process, once a certain stimuli threshold was reached, regardless of state or time of immobility, the fish regained wakefulness instantaneously (Fig. 8). This is a key observation, especially when wanting to induce external stimuli to test arousal thresholds.

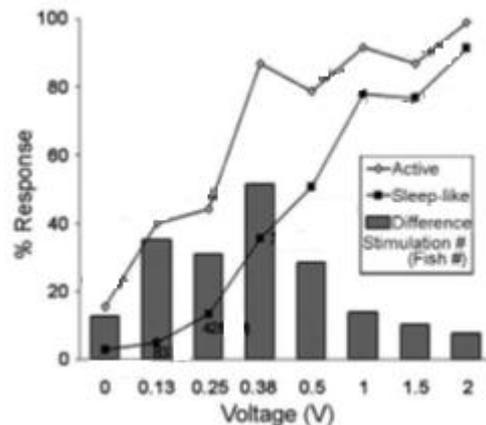


Figure 8. The lines depict the percentage of electrical stimuli (in Volt) eliciting responses (movement) in both active and sleep-like (prior inactivity of 6 seconds) fish. The bar graph depicts the differential response in active and sleep-like fish to increasing Volt. Largest difference is at 0.38 Volt. – *Yokogawa et al. 2007*.

At electrical stimulation level of 0.38 Volts and higher, all fish that were applied with stimulus reacted (Fig. 8). Thus the maximum electrical stimulation voltage is 0.38 Volts. This is true for both day and nighttime stimulations.

They defined the minimum time for the fish to be immobile, in order to distinguish sleep from mere inactivity, by using the receiver operator curve (ROC analysis) of the results of the electrical stimulation experiments. This revealed that the zebrafish must be inactive for 6 seconds to reveal unresponsiveness (movement is the expected response) to a stimulus of 0.38 volts (higher arousal threshold). If the fish were inactive for a longer period than 6 seconds, then its’ arousal threshold would increase as well. Thus the fish must be at least six seconds inactive, before they were

recognized to be sleeping. But, in order to be of complete certainty, they must consider using EEG measurements to reveal brain waves and patterns.

The type of stimuli used to determine arousal threshold seems to be of importance. *Yokogawa et al. 2007* stimulated the zebrafish with small amount of electricity. They observed that at higher voltages all fish responded immediately, even those who had been showing behavioral signs of sleeping. This provides us with a maximum electrical stimuli output to use for further arousal threshold measurements.

Yokogawa et al. 2007 stated that the adult zebrafish is highly sensitive to light arousal, and remained in continuous activity for seven days under continuous light exposure. This uncovered direct interaction of light with the arousal system of the fish (*Yokogawa et al. 2007, Zimmerman et al. 2008*). Sleep reintroduced itself after 8 days of continuous light; this confirms what *Hurd & Cahill 2002* had established: “that only a portion of adult fish displayed detectable circadian activity rhythms under constant light”.

Zhadanova et al. 2001 studied the sleep like state in zebrafish. They found highly diurnal activity and a nocturnal decrease in motor activity together with an increase in **arousal threshold**.

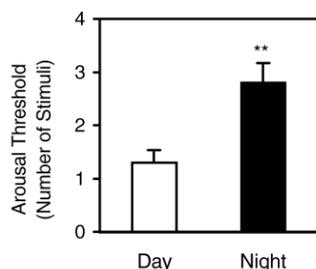


Figure 9. Arousal threshold (as number of stimuli) during both day and night in zebrafish, $n=20$ for each group. There is a significant increase in applied arousal threshold stimuli during the night. – *Zhadanova et al. 2001*.

They used the same arousal threshold method as *Duboué et al 2011*.

This observation persisted even when the zebrafish were exposed to constant darkness (*automatic image analysis system*) which they measured with backlighting from an infrared camera onto micro-plates. The data was converted and stored as distance traversed of every fish at every 15 seconds.

They concluded that daytime rest deprivation (caused by continuous vibration within the tank) during the day did not significantly affect the amount of resting behavior thereafter, although they did spot a decrease in motor activity and a slight elevation in arousal threshold. Thus the fish had higher sleep intensity.

Perhaps if they had used EEG measurements during this experiment they might have seen whether these observations coincide with an intermediate conscious sleep state (Fig. 1). If that would be true, then the zebrafish would rely on sleep rebound, even after daytime rest deprivation.

Cirelli & Tononi 2008 discussed whether sleep was essential and searched for sleep studies done on various animals, including fish. The zebrafish have been observed to rely on homeostatic sleep regulation (*Zhadanova et al. 2001, Prober et al. 2006*); they show an increase in sleep time and arousal threshold after 6 hours of sleep deprivation (*Prober et al. 2006, Yokogawa et al. 2007*).

With light arousal threshold stimulation the zebrafish show no need for rebound sleep (*Zhadanova et al. 2001, 2008*), and in fact, they reduced their sleep time to approximately 20% of their original (pre-light-stimulated) duration. They continue at this highly decreased sleep duration for up to 8 days after initial light stimulation (*Yokogawa et al. 2007*).

WRASSE

Hur et al. 2012 studied the wrasse (Fig. 10) and they found that most of them exhibit a distinct daily rhythm in activity, depending on ocean-bottom properties. They observed wrasses emerging from sand in the morning, remain active throughout the day and subsequently bury their selves in the 'sandy' ocean-floor for the night.



Figure 10. *Thalassoma lunare*, moon fish, this is a typical wrasse and was studied by Hur et al. 2012. (Source: Leonard Low, Australia)

They noted the following behavioral criteria; a **typical place/nest & typical time of day/circadian rhythm** and **immobility**.

The wrasses even continued this behavior when kept under constant light conditions. The wrasse continued its circadian rhythm perfectly, burying itself in the sand at 'biological' nighttime, even though it was light outside. Somehow the pathway to remain its' circadian rhythm is stronger than the visual input of light. It is unfortunate, however, that the research did neither involve arousal threshold measurements nor tests for the presence of a rest/sleep rebound after rest/sleep deprivation. It could be for other reasons than sleeping that the animal buries itself in the sand, perhaps predatory reasons, or prey unavailability. In any case, the first observations of the wrasse have been made, clearly depicting a strong rhythm and placement, but nothing on the individual itself at that specific time. One could not conclude that the fish is in fact sleeping.

CORAL REEF FISH

Goldshmid et al. 2004 studied sleep in coral reef fish (*Dascyllus marginatus* Fig. 11, *D. aruanus*, and *Chromis viridis*). These species had been observed swimming continuously throughout the night within their coral host (**typical place**).

The authors concluded this type of 'activity' to be none other than 'sleep-swimming'.

There are several benefits for both the fishes and corals with their continuous swimming during the night. Through their swimming they provide oxygen rich air and nutrient upwelling to the coral during the most difficult time of day, night-time (Nilsson et al. 2007). During the night the coral needs oxygen,

Because the corals have vast and complicated structures, they tend to block out oncoming flow of oxygen 'rich' water. After a night of oxygen consumption by host fish and their own oxygen consumption, they would be left with anoxic pockets within the coral reef. The continuous activity of the fish during the night prevented such a thing from happening, and thus they have a symbiotic relationship.



Figure 11. *Dascyllus marginatus*, coral reef fish species, studied by Goldshmid et al. 2004. (Source: Fishbase.org)

The article was quick to judge their observed nightly activity as 'sleep-swimming'. First, they compared swimming speed of the fish inside (at night) to that of outside (daytime) the coral (Fig. 12). Second, the images were analyzed,

determining their fin movements and overall speed. And third, they compared the two observations (averages of swimming speed/behavior in- and outside the coral), and stated that there was a significant difference between the two.

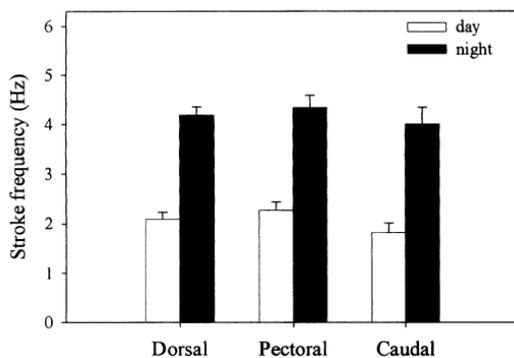


Figure 12. Stroke frequencies in Hz of the dorsal, pectoral and caudal fins of the *D. marginatus* inhabiting the coral *S. pistillata*, during the day ('normal swimming' outside of coral) and night ('sleep swimming' inside the coral). There was a significant difference between the stroke frequencies. - Goldshmid et al. 2004

The fish swimming inside the corals during the night had decreased fin movements, as well as speed. Hence, so was concluded. the fish swimming in the corals must be sleeping.

In this case, they compared two highly different situations, a fish swimming during the day with unlimited swimming space, compared to one at night with a very restricted swimming area. And just because its movements are slower or less frequent, does not automatically mean that it is sleeping. These two situations are, in fact, far too different from one another, and could/should not be compared with each other. If they had used a different method to determine sleep in these fishes, for instance the stimuli method used by Duboué et al. 2011, they might have been able to conclude whether the fish had increased arousal thresholds and slower swimming speeds.

For now I would prefer to call their observation 'night-swimming' instead of 'sleep-swimming'. The study will have to be

repeated and checked with more appropriate and established sleep criteria, before anything else can be concluded from it.

THE SCHOOLING TILAPIA

An older study regarding sleep in fish by Shapiro & Hepburn 1975, revealed resting behaviors in the schooling *Tilapia mossambica* (Fig. 13). They categorized two types of behavioral patterns during the day:

1. *State A*; very active swimming phase (more than ten fish displacements/min). Eye-movement.
2. *State B*; More passive phase of 3 or fewer displacements/min with less than 3 fish resting on the bottom of the tank at any time. Lower respiratory rate than state A. Eye-Movement.
3. *State C*; even more passive than *State B*, during which 12 of 15 fish were observed to rest on the bottom (**Typical posture**). Respiratory rate equal to *State B*. No eye-movement

During *state A* the fish exhibit larger respiratory activity (determined by gill movement), than those in *state B*. These two categories cycled throughout the light period (One cycle lasted on average thirty minutes, with twenty minutes of *state A*, combined with ten minutes of *state B*). They did find that this cycle was easily interrupted by environmental stimuli (such as feeding).

	State A	State B	State C
Threshold (V)	5.1±1.2	4.8±1.1	9.1±1.6

Table 2. Threshold measurements in voltages, state C required the largest electrical stimulation, thus has the largest arousal threshold. State C is significantly higher than both A and B. There is no significant difference between State A and B - Shapiro & Hepburn 1975

The onset of darkness increased the presence of *state B*, the fish became more passive and subsequently made way for a third state, *state C*.

State C was also accompanied by an increase in **arousal threshold** (response time to feeding and higher level of electrical stimuli needed for response) as well as decreased activity (**quiescence**) (see Table 2.).

Arousal threshold was measured with 2 wire leads with metal plates at their ends placed diagonally at opposite sides of the tank. Responses to electrical stimuli were; gulping & opening and closing of the mouth. They concluded to have found sufficient behavioral criteria to announce the Tilapia to be sleeping.



Figure 13. *Tilapia mossambica*, a schooling cichlid studied by Shapiro & Hepburn 1975. (Source: fishbase.org)

FRESHWATER YELLOW PERCH

This fish (*Perca flavences*, Fig. 14) was studied by Helfman 1979. They found the Perch to show an **immobile** state/certain **posture** during the night and moves towards the bottom of the ocean to rest. They have made no further attempt to measure **arousal threshold**. Other than revealing a strong reaction (fish jolted away back to darkness) when their flashlight beams crossed their paths. The fish were observed to move away from the beam and settle back on the bottom again, back to their 'sleeping' **posture** (Helfman 1979).

So far, most studies have been performed on sleep in the zebrafish, considering sleep in fishes, the zebrafish is the sleep study model

of preference. The zebrafish is are hardy species and easily bred in aquaria, their genome has been fully sequenced and their embryo's develop outside of the body, thus easily manipulated (Dahm 2006)



Figure 14. *The Freshwater Yellow Perch (Perca flavences)* studied by Helfman 1979. (Source: Fishbase.org)

Even with all the sleep studies performed on zebrafish, the amount of behavioral criteria used varies strongly (Table 3) The study of Yokogawa *et al.* 2007 relied on most of the mammalian sleep criteria; they concluded that the zebrafish is very likely capable of sleeping.

Either sleep studies have to enhance equipment and start with EEG measurements in fish, or they need to increase attempts to exploit all mammalian sleep criteria for their subjects. Without either one of these, the outcomes of the studies are inconclusive. Though EEG recordings alone will not provide conclusive results, combined with behavioral sleep criteria, they will most likely prove to be very effective.

SLEEP EVOLUTION

It is thought that sleep was not always 'necessary' or even present within the animal kingdom. It is hypothesized that evolution of more complicated life and increasing presence of specialized brain regions, along with multi-functionality of those regions, pushed the animals' to undergo periods of sleep, in order to keep up with internal complications (Reebs 1992, Kavanau 2004,2006).

Article	Species studied	Sleep criteria used	Conclusion article
Duboué et al. (2011)	Cavefish (<i>Atyanax mexicanus</i>)	Immobility Arousal threshold	Immobility > 60 seconds considered to be sleep
Goldshmid et al. (2004)	Damselfish (<i>Dascyllus marginatus</i>) Humbug damselfish (<i>D. aruanus</i>) Blue-green damselfish (<i>Chromis viridis</i>)	Fin movement Swimming speed Typical place	Significantly lower speed and less fin movement considered to be sleep. Show this behavior in their host coral, thus have a specific 'resting' place.
Helfman (1979)	Freshwater Yellow Perch (<i>Perca flavences</i>)	Posture Immobility Arousal threshold Typical place	Yellow Perch sleep and are 'easily aroused' by light, after which they quickly continue to sleep.
Hur et al. (2012)	Three-spotted wrasse (<i>Halichoeres trimaculatus</i>)	Typical place/nest Circadian rhythm Homeostatic regulation	Wrasse bury themselves in the sand before night falls (even in experimental complete light cycle), they are considered to be sleeping
Shapiro & Hepburn (1975)	Mozambique tilapia (<i>Tilapia mossambica</i>)	Swimming speed Arousal threshold Eye movement Quiescent Typical posture	Active swimming (>3 fish lengths/min), they are awake. Response time to adding of food, and electrical stimuli. Response: gulping or opening & closing the mouth.
Yokogawa et al. (2007)	Adult Zebrafish (<i>Danio rerio</i>)	Posture Immobility Quick reversibility Arousal threshold Typical place/nest Homeostatic regulation Circadian rhythm	Name this method the ASFRS for adult zebrafish, if they show these sleep criteria, they are considered to be asleep.
Zhadanova et al. (2001)	Zebrafish (<i>Danio rerio</i>)	Posture Homeostasis Circadian rhythm Motor activity Arousal threshold	Clear diurnal activity, preserved even after continuous darkness. Nocturnal decrease in motor activity & increase in arousal threshold.

Table 3. Summary of fish sleep studies, the criteria used by the articles to assess whether the fish was sleeping, their conclusion from it, and my opinion on said criteria for sleep determination. The sleep criteria in **bold** lettering are those who aren't present in the mammalian behavioral sleep criteria, the authors created new criteria for sleep determination. The Yokogawa et al. 2007 article is the one most complete with mammalian sleep criteria. They studied the adult zebra fish, and as the table shows, the zebra fish has been studied most within the fish sleep research field.

Life started with animals that led 'simple' lives. They had few to very little needs to process and store long-term memories and relied on simple tasks that they performed almost continuously (Allison & Van Twyler 1970, Kavanau 2004, 2006).

But then, life started to become more complex, it evolved, and the need for Detailed Focal Vision (DFV) increased, DFV was then used for either prey spotting, or predator awareness (Reebs 1992, Kavanau 2006).

Interestingly, sight has been given the front seat when considering the necessity of sleep. Other sensory input methods receive

far less attention, though they might as well be large contributors to increasing need of sleep. Other sensory input is e.g. smell, sound or echolocation. These are also high maintenance and specialized parts for prey and predator spotting.

DFV (as well as the other sensory input) continued to grow, along with the growth of the brains capacity to process and store new events. In full motion, the multi-functionality of the brain started to clash with the animal's other processes, for instance detailed locomotion coordination (Kavanau 2004).

The obvious advantage of DFV, was allowing the animal to capture a lot of environmental information, but this increase in information had to be processed as well, next to the ability for the animal to react to sudden changes. Memory storing and quick reactions to environment occur within the same brain region, causing them to clash (Kavanau 2006). In this case the animal would want to rely on instantaneous reaction, more so than on memory saving, the first option immediately increasing survival and the second mostly for future survival. So memory storing was labeled as a necessary process, but to be done when the animal was in a secure environment without being disturbed, or in need of instantaneous reacting to the environment. These conflicts, both part of the animals' survival, were alleviated by evolving the first steps for 'sleep' (Kavanau 2006). This process most likely started with 'restful waking', followed by 'primitive sleep' and topped off with fully developed sleep (including two new states, REM and NREM (Fig. 1), so far, discovered in mammals and birds) (Kavanau 2004, 2006, Rattenborg 2006).

Duboué et al. 2011 concluded a decrease of sleep in the blind Mexican cavefish (Fig. 6, Table 1.) yet still sleep remained present in the blind cavefish. If sleep were in fact only present due to the presence of complex eyes, then the blind cavefish would have no need to sleep at all.

This emphasizes that there is more to the reason for sleep than sight sensory input. Therefore other sensory input should be evaluated as well (e.g. sound, smell and echolocation).

Either DFV can only explain sleeping behavior in certain 'sleeping' animals or the blind cavefish is in transition state, and will be expected to lose sleep all together (*Duboué et al. 2011*). Nonetheless, we can't

conclude whether DFV is the driving force of sleep evolution (*Rial et al. 2007*).

DISCUSSION

Sleep identification requires many steps, some of which have been ignored by current sleep studies. Few studies have regarded all mammalian behavioral sleep criteria; some have added additional criteria to enable sleep recognition (Table 3).

Most likely in all studies, the fish were inactive for an unspecified period of time. However, as mentioned before, inactivity is not necessarily sleep.

There are many researchers under the assumption, that all fishes must sleep, but do they? I would argue against the sleep-swimming coral-reef fish, not because the fish must be inactive when sleeping (even though mammalian sleep criteria argue against that) but because the authors did not provide sufficient information to determine whether they were actually sleeping. No arousal thresholds were measured, no sleep rebound study. In fact, nothing of the mammalian sleep criteria was used to recognize them to be asleep.

It might be that the provided behavioral mammalian sleep criteria are insufficient for sleep recognition in fishes. We have been preoccupied at finding sleep in more recent evolved mammals, and forgot to look at the basic animals, which seem to show signs of sleep as well. If ancient fish sleeps, than that means something in recent evolved animals and their sleep as well. More ancient animals usually have more basic lifestyles, it is expected that these animals easier to understand, and therefore easier to perform experiments with.

Another important step would be to measure EEG in fish. These two combined, would be able to reveal whether that certain

species of fish is sleeping or not. Once it is clear that they sleep then we might expand our search as to the reasons for it, as well as how this has changed over generations and decades. If future research continues with mammalian behavioral sleep criteria, the emphasis should be on the increase in arousal threshold. Perhaps they should even combine oxygen consumption rate and heart rate, assuming that a sleeping/resting fish would have decreased oxygen consumption and a lower heart-rate (Roche 2013). That would only confirm a difference between activity and rest, but combined with the other behavioral criteria (e.g. arousal threshold) would give an extra dimension to overall sleep determination. Until now, we can only say that it is very plausible (considering the behavioral criteria) that the fish is capable of sleep.

CONCLUSION

Do fish sleep?

Researchers have been very preoccupied in finding reasons as to why fish sleep, that they completely lost track of the identification process needed to establish sleep in fish.

Many studies have either, only taken a few behavioral criteria of sleep into account, or made up complete different criteria, which on hindsight; do not make much sense at all. So far, we can't say with complete certainty that fish sleep. The evidence collected so far does strongly suggest that fish sleep, but it remains inconclusive.

So in answering the question: 'do fish sleep?' there is no conclusive 'yes', yet. However fish sleep research has grown a lot and shows increasing possibilities for sleep identification.

I recommend sleep researchers to increase the amount of fish sleep studies. As well as incorporating the use of EEG, in order to establish a 'numerical' difference between their wakefulness and supposed 'sleeping'.

Once we are able to conclude sleep in fishes, we might think of implications for more recent evolved animals and their need for sleep. For now, instead, there should be a focus on a new sleep study motto: "the study subject is awake, until proven sleeping".

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