



## Description of the feeding rhythm of cone snails in French Polynesia

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### ABSTRACT

The Conidae is a family of marine gastropod molluscs that includes carnivorous and venomous species. The venom they produce is remarkably diverse and has recently drawn much interest from a pharmacological perspective. To respond to this growing interest, a better understanding of the ecology and biology of the species is required. Only a few studies have examined the feeding behaviour and the trophic specialization of these species in detail. In this study, we investigated diet through hunger and satiety experiments of eight species of cones that occur in French Polynesia and that present different feeding modes (piscivorous, molluscivorous or vermivorous). Monitoring behaviour of cones during fasting periods demonstrated a correlation between increased hunger and nocturnal cone activity, mainly for piscivorous species, while the molluscivores did not show this correlation. In the study on satiety, we defined an average frequency of feeding for cone species that were fed prey *ad libitum*. Finally, this study provided indications that will help to define an equilibrium feeding frequency in further studies involving the cultivation of cones.

### INTRODUCTION

Conidae is a taxonomic family that was described by John Fleming in 1822. Composed of 109 genera and more than 650 species (World Register of Marine Species), this family of marine gastropod molluscs includes only carnivorous and venomous species that are generally known for their toxicity. Mainly found in warm and tropical waters worldwide, cone snails are most diverse in the Indo-West Pacific region (Rockel, Korn & Kohn, 1995). This family is represented by 110 species in French Polynesia (Tröndlé *et al.*, 2020; Richard & Rabiller, 2021) and can be found in a wide range of habitats, from the intertidal zone to deeper areas, living on sand, among rocks or on coral reefs (Rockel *et al.*, 1995).

Appearing about 56 million years ago (Richard & Rabiller, 2021), this family is among the most recent in the molluscan world and the most evolved in the gastropod class. As they are carnivorous, cones must hunt their prey and as such have developed efficient hunting tools such as a harpoon-like radula and a cocktail of potent venom peptides called conotoxins to overcome their natural slowness. Each species of cone typically possesses an average of 100–200 conotoxins as potential pharmacological targets (Peng *et al.*, 2016). These conotoxins comprise an assembly of small peptides of 12–30 amino acids in length (Olivera, 2002) that are bound to a wide range of membrane proteins (Wang & Chi, 2004), which makes them interesting to the medical field. They have already shown medical properties such as antiepileptic, antinociceptive, as well as cardio- and

neuroprotective activity. Conotoxins are also increasingly useful as research tools for the study of cancer and for neuromuscular and psychiatric disorders (Han *et al.*, 2008; Twede *et al.*, 2009).

The present work is part of a larger research programme that aims to develop a long-term management strategy for cones in French Polynesia. The present study aims to improve general knowledge about the feeding requirements for cones, which will provide important information for long-term rearing in aquarium facilities. We sought to define a nutritional balance such that the cones did not lack food, but that they remained hungry enough to enable the sampling of their venom (milking).

To meet these objectives, we conducted two experiments. The first aimed to investigate fasting and its effect on the behaviour of Conidae at night. Monitoring of cones at night provided information that allowed for the rhythm of activity for each species to be determined and allowed us to identify distinctive behaviour that could be interpreted as a sign of hunger. As the behaviour of cone snails is relatively understudied, apart from a few articles in the literature on predation in a few species (Stewart & Gilly, 2005; Olivera *et al.*, 2015), this study will provide additional data on the nocturnal behaviour of cone snails. Our experiment tests the hypothesis that Conidae behaviour changes with increasing hunger. The second experiment focuses on the natural feeding frequency of cones in the constant presence of prey. This experiment allowed us to adjust and optimize feeding rhythm in an aquarium setting. This

**Table 1.** Details of cone snail species studied including diet, number of individuals studied, origin of studied specimens (i.e. collection locality) and prey.

Species	No. of individuals	Origin	Prey
<b>Molluscivorous</b>			
<i>Conus textile</i> Linnaeus, 1758	8	Mooréa	Conidae: <i>C. pulicarius</i>
<i>C. canonicus</i> Hwass, 1792	12	Nuku-Hiva	Conidae: <i>C. pulicarius</i>
<b>Piscivorous</b>			
<i>C. bullatus</i> Linnaeus, 1758	10	Nuku-Hiva	Apogonidae
<i>C. catus</i> Hwass in Bruguière, 1792	8	Nuku-Hiva	Poeciliidae
<i>C. gauguini</i> Richard & B. Salvat, 1973	5	Nuku-Hiva	Apogonidae
<i>C. striatus</i> Linnaeus, 1758	8	Mooréa	Apogonidae
<b>Vermivorous</b>			
<i>C. conco</i> Puillandre & Cie, 2015	12	Nuku-Hiva	Nereidae
<i>C. imperialis</i> Linnaeus, 1758	11	Nuku-Hiva	Amphinomidae

experiment tests the hypothesis that an average feeding frequency can be defined in the case where prey is constantly present.

## MATERIAL AND METHODS

### Collecting, housing and feeding

The species used in this study were selected based on their diet. We targeted piscivorous, molluscivorous and vermivorous species to best represent cone snail diversity. Overall, eight species of cone snails were used in this study. Six species were collected in Nuku-Hiva (Marquesas Islands, French Polynesia) in November 2020, and two were collected in Mooréa (Society Islands, French Polynesia) in February 2021 (Table 1). Each specimen was placed in a 3-l individual aquarium and fed once a week with specific prey after their appetite was tested with a variety of potential prey. To approximate the original habitat, a 2-cm layer of a mixture of fine and coarse sand (0.063 < size in cm < 0.5) from the Mooréa lagoon was added to the bottom of the aquarium. *Conus imperialis* individuals did not show signs of burrowing, so the sand was removed from their aquaria to facilitate cleaning. The aquaria were continuously supplied with seawater from Opunohu Bay (Mooréa, French Polynesia) via the research centre's running seawater system and maintained at a temperature between 26 and 28 °C with a 12:12 light/dark cycle using artificial lights.

The cone snails were each provided with live prey in their aquaria, which allowed them to hunt in a more or less 'natural way'. As the literature did not provide enough information concerning specific prey preferences, we selected prey based on diet, size and prey availability in the natural environment. For piscivorous species, while cones have demonstrated the ability to engulf prey even larger than themselves (Richard & Rabiller, 2021), we decided to limit prey length to the shell length of the cone. Among the fish present in Mooréa, Apogonidae and Poeciliidae were considered good candidates based on interest shown by the cones and because they were abundant enough to be collected in Mooréa. For molluscivorous species, Cypraeidae as well as other

Conidae smaller than the studied cone species were used as prey. The molluscivorous species showed a particular attraction for *Conus pulicarius*, a species that is easily found on the sandy bottom of Mooréa's lagoon and that was selected as the main prey. The vermivorous species are known to preferentially eat polychaete annelids. *Conus imperialis* exclusively consumes members of the Amphinomidae (Kohn, Nybakken & Mol, 1972), while *C. conco* consumes polychaetes from the family Nereidae. The worms supplied range in size from 6 to 12 cm; however, the vermivorous species sucked and then cut the worm to the desired length.

### Investigating foraging activity to determine a hunger index

This experiment studied the nocturnal behaviour of cones during a fasting period. The experiment took place in March 2021 when eight individuals of six different species of cones were studied simultaneously: two piscivorous (*C. catus* and *C. bullatus*), two vermivorous (*C. imperialis* and *C. conco*) and two molluscivorous (*C. canonicus* and *C. textile*). All cones in each of the experiments were fed at the same time, with prey adapted to their diet, to bring them closer to a state of satiety. Next, the cones were fasted for a period of 8 days. During this period, the nocturnal activity of the cones was monitored using time-lapse photography.

Time-lapses started every evening at 18:00 and ran until 06:00 the next day with a photo taken every 3 min, for a total of 241 photos for each series of four aquaria per night. For each species, two sets of four aquaria were used at the same time to conduct the experiment on eight individuals of the same species simultaneously. A camera was placed in front of each series of four aquaria for the time-lapse image capture.

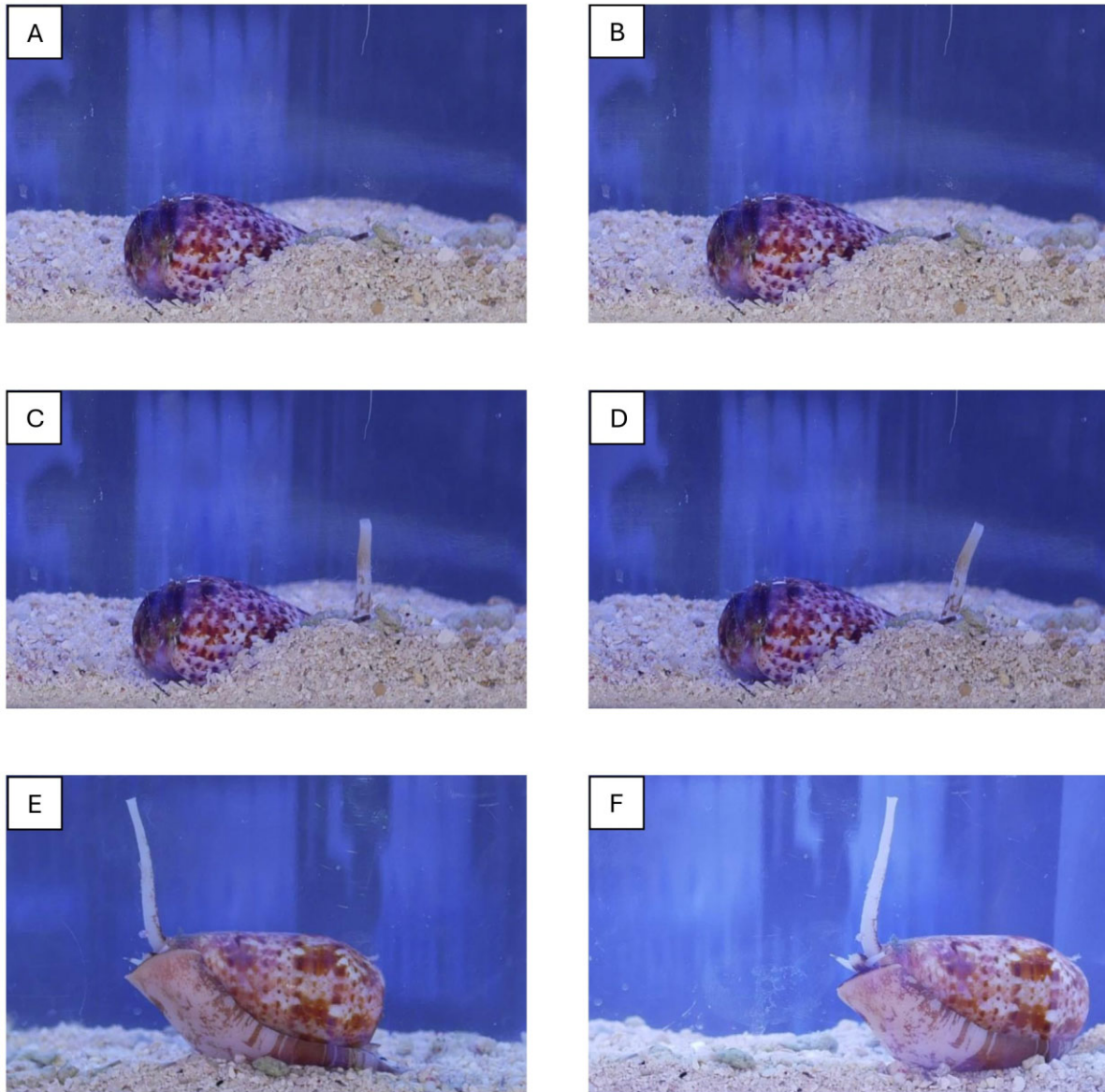
Modified aquaria, called "photo aquaria," were used in this experiment. These are individual aquaria of 2.3 l filled with a 2-cm layer of sand on the bottom to mimic their natural habitat (Kohn & Nybakken, 1975). These aquaria have right angles to avoid distortion of the image, and the sides were covered with a dense plastic plate to avoid visual interactions between cone snails in neighbouring aquaria. One hour before the beginning of time-lapse photography, the individual cones were placed into the photo aquaria to give them time to acclimate to their new aquaria. At the end of each experiment, the cones were returned to the original aquaria where they were maintained.

The first analysis of the photos consisted of taking each photo, taken at 3-min intervals, and providing a detailed description of the cone's movement between two successive photos. Based on earlier and initial observations of the cones, we defined three types of movement that we called states (Fig. 1):

- (1) 'Rest' is characterized by a cone that is completely immobile for two consecutive photos (i.e. no movement of the shell or the siphon between two photos taken 3-min apart), with a retracted or barely emerged siphon.
- (2) 'Passive search' is characterized by no movement of the shell, but with a siphon that is clearly visible and that extends from the shell in different positions, as observed between two successive photos.
- (3) 'Active search' is characterized by a displacement of the shell between two photos and a clearly visible siphon.

The number of photos representing each state was then counted and transformed into a percentage to quantify and compare the three states over time.

For this experiment, we expected that individual cones of all species would exhibit an increase in foraging (i.e. increase in 'active search', category 3) through time during the nocturnal phase. This foraging could translate into a greater percentage of active time and a reduced resting time. A correlation test was performed to evaluate the trends in activity across the three states characterized above. Since the data did not provide a normal distribution, a Kendall



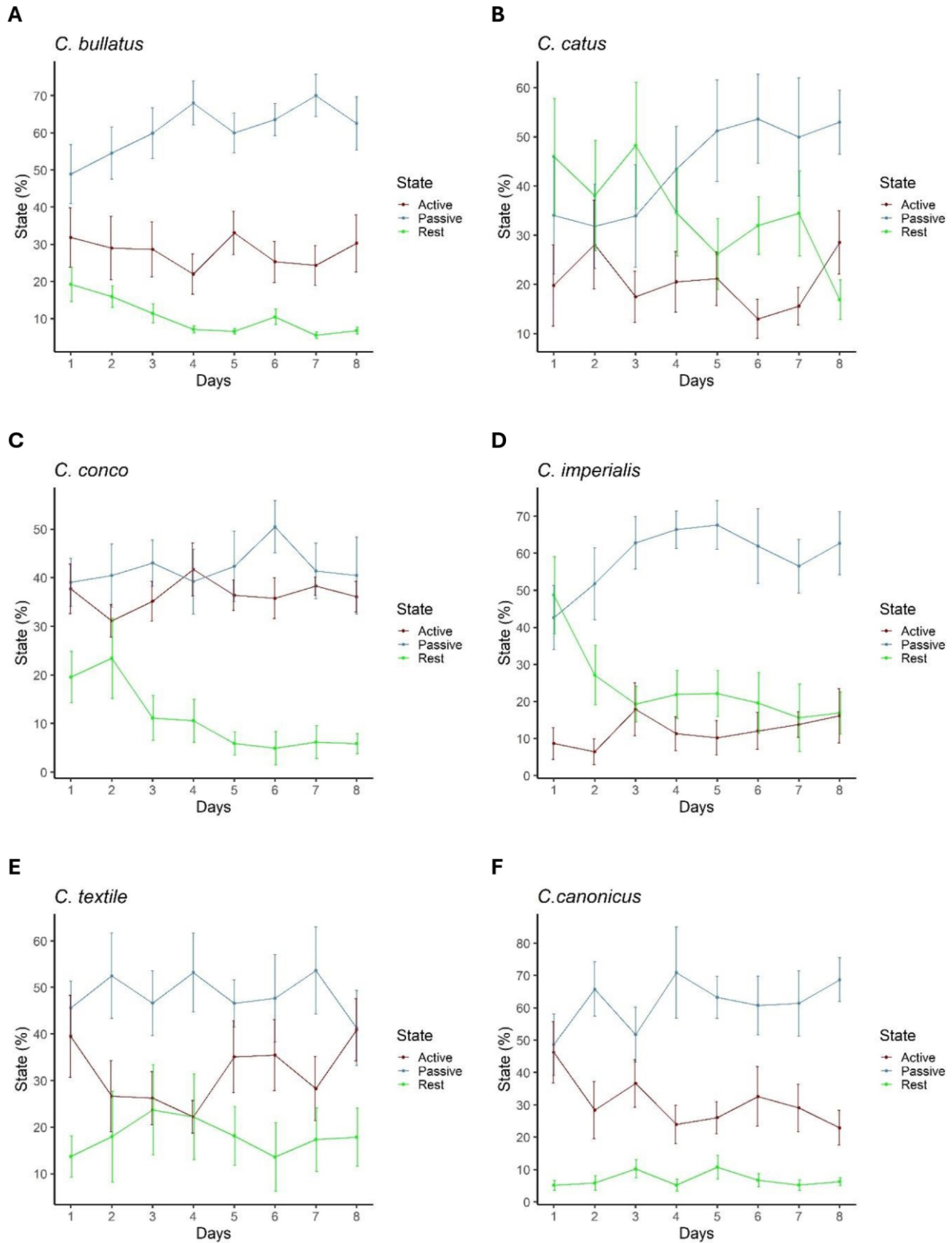
**Figure 1.** Different states of *Conus bullatus*. **A, B.** ‘Rest state’ with no movement of the cone. **C, D.** ‘Passive search state’ with movement of the siphon. **E, F.** ‘Active search state’ with movement of the entire individual.

correlation was chosen via the `cor.test` function of the `stats` package in the R environment R 4.2.2 (R Core Team, 2022). The  $P$ -values and Kendall’s tau were derived from the results of this function. The  $R^2$  was obtained by performing the regression between each state and the fasting days with the `lm` function of the `stats` package.

#### *Determining the natural feeding frequency of cone snails*

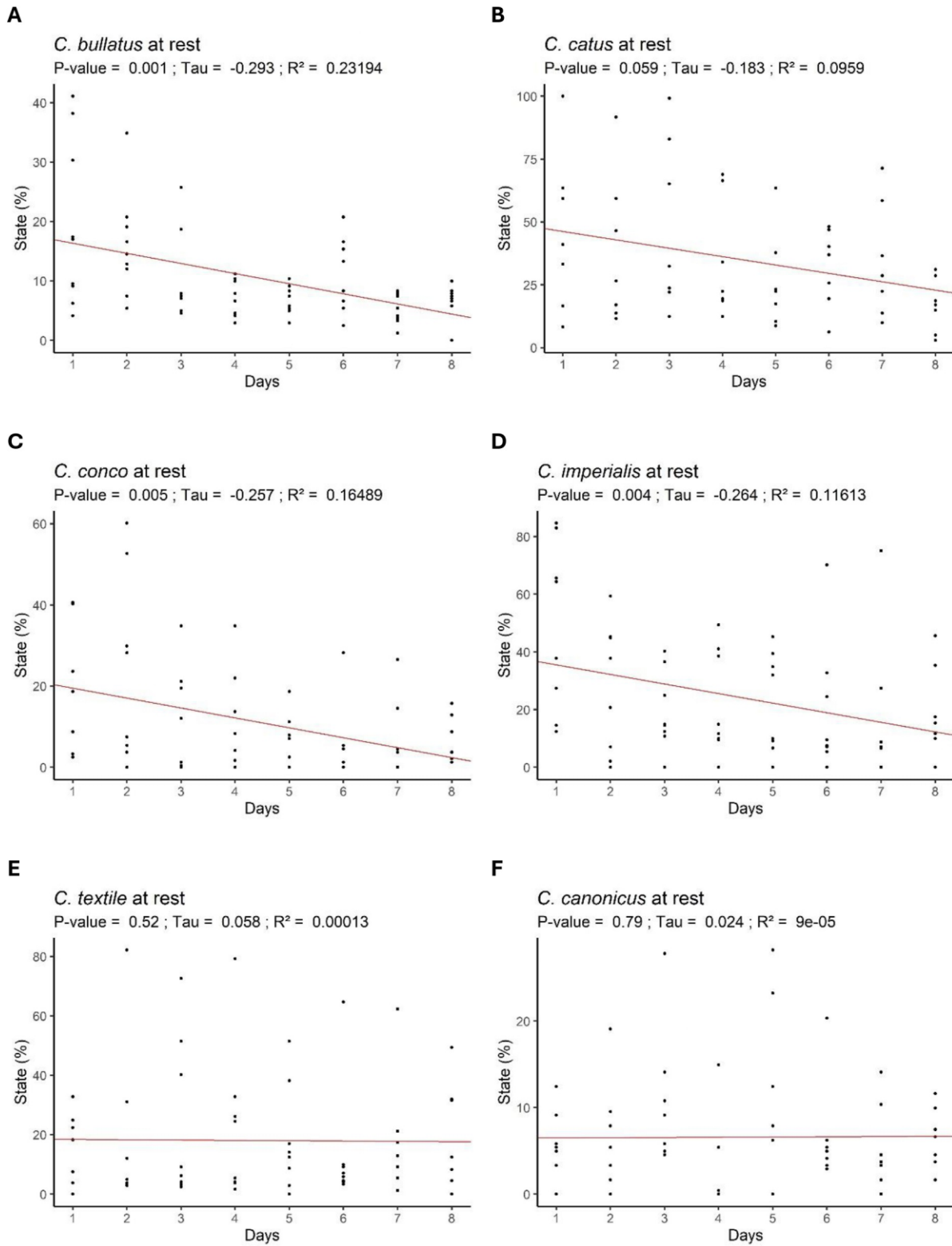
This second experiment was conducted after the first, in April 2021. Six different species were studied, including three piscivorous (*C. bullatus*,  $n = 10$ ; *C. gauquini*,  $n = 5$ ; and *C. striatus*,  $n = 10$ ), one vermivorous (*C. imperialis*,  $n = 11$ ) and two molluscivorous (*C. textile*,  $n = 7$ ; and *C. canonicus*,  $n = 12$ ), giving a total of 55 individuals. These species were selected because they represent the three major diets found in cones and because the prey that were required to feed them were available in quantities large enough to meet the needs of this experiment.

The experiment consisted of monitoring the feeding frequencies of the different species of cones. First, all specimens were fasted for 1 week in order to start the experiment with a very low and equal level of satiety. On the first day, selected prey, adapted to the diet of each species, was placed in each aquarium. At the start of the experiment, and at each 12-h interval, feeding individuals were recorded and the prey was replaced. Relative prey mass (RPM), which is prey mass/cone mass  $\times 100$ , is an important factor in food ecology. A variation of the RPM has been shown to result in a modification in satiety for some species (Nielsen, Jacobsen & Wang, 2011). To avoid this variation, an RPM of 5% was defined for all of the selected prey. In the absence of a sufficient range of prey weights, the prey selected for the experiment were those that were closest to the 5% RPM. For vermivorous species, this was different, because despite the 5% RPM that was provided, some cones had a lower food intake as they cut the worm and only consumed part of it.



**Figure 2.** Set of graphs showing the percentage of time spent (mean  $\pm$  standard error) in each state per night for each cone species. **A.** *Conus bullatus*. **B.** *Conus catus*. **C.** *Conus conco*. **D.** *Conus imperialis*. **E.** *Conus textile*. **F.** *Conus canonicus*.





**Figure 3.** Set of graphs showing the correlation line of the Kendall's test between the percentage of time spent resting and the number of fasting days for each cone species. **A.** *Conus bullatus*. **B.** *Conus catus*. **C.** *Conus conco*. **D.** *Conus imperialis*. **E.** *Conus textile*. **F.** *Conus canonicus*.

This experimental design allowed us to determine the time intervals between two successive feedings for each individual cone. However, to remove the influence of day and night on the feeding of snails, the number of individuals that fed per time interval was summed up to provide a uniform and unique 24-h period. From this, the data were then transformed into frequencies. To compare the times between feedings for the different species, the normality of the data was tested using the `shapiro_test` function of the `stats` package. Since the data did not provide a normal distribution, a nonparametric Kruskal–Wallis test was performed using the function `kruskal_test` from the same package.

## RESULTS

### *Investigating the foraging activity to evaluate a ‘hunger index’*

The graphs provided in Figure 2 show the percentage of time spent by the cones in each of the three ‘states’ for each night and their variation throughout the fasting. The results of the activity curves indicate that for *Conus textile*, *C. canonicus*, *C. bullatus* and *C. conco*, most of the night-time activity corresponded to passive searching with values between 40 and 70%, followed by active searching with values between 23 and 48% and resting with values between 5 and 25% (Fig. 2). In the case of *C. catus*, rest time appeared greater than passive search for the first 3 days and greater than active search over 7 days, with a gradual decrease from 46 to 20%. For *C. imperialis*, the time allocated to rest was greater than active search on the first day of fasting with a value of 49%, which corresponds to the highest rest time observed throughout the study. Resting time then decreased progressively to reach a plateau between 15 and 22%. With values of 46 and 49%, *C. catus* and *C. imperialis* were the two species with the highest resting time on the first day of fasting.

For *C. textile*, *C. canonicus*, *C. bullatus* and *C. conco*, the percentage of time spent as ‘resting’ decreased gradually during the first few days and then seemed to reach a plateau for vermivorous and piscivorous species. This plateau was reached differently according to the species on days 3, 4 or 5. In *C. catus*, the resting time started to decrease on day 3 and through day 8, no plateau was reached. The decrease in resting time seems to be compensated by the increase in passive search that also evolved, but in this case, began to increase from day 3. However, even though the resting time did not reach a plateau for *C. catus*, the latter clearly reached the passive search time trend on day 5. For molluscivorous cones, there was no decrease with respect to the resting time, which remained more or less unchanged in both species throughout the experiment. However, for *C. canonicus*, a different pattern emerged as it was the only species for which a decrease in active search time was recorded. Further, this decrease seemed to be compensated for by an increase in passive search time. The results of the different states for *C. canonicus* did not show any distinguishable trend; all curves were relatively constant, with rest ranging from 13 and 24%, passive search ranging from 45 and 54% and active search ranging from 22 and 40%.

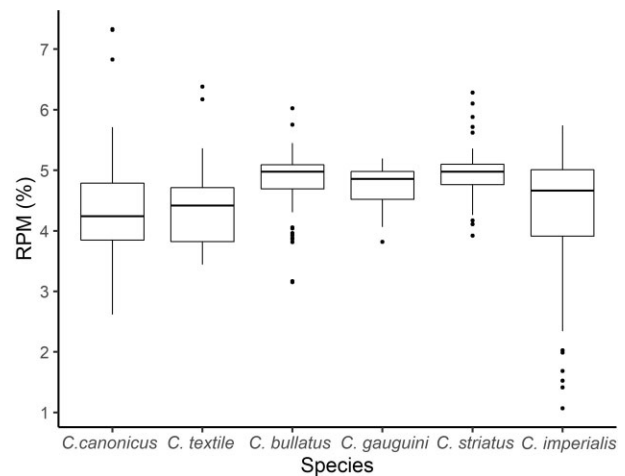
Interestingly, for all species, the standard error was on average 6.9% and varied from 0.8 to 12.9%, with the lowest values obtained for the resting curve of *C. bullatus* and the highest values for the resting curve of *C. catus*. Overall, the observed trends were fairly similar among the eight individuals of the same species.

Kendall’s correlation analyses (Fig. 3, Table 2) indicate that there is a significant relationship between the decrease in time spent resting and the number of days fasting for some species. This is especially the case for *C. bullatus*, *C. conco* and *C. imperialis*, with *P*-values of 0.001, 0.005 and 0.004, respectively. The results for *C. catus* show a similar pattern and a *P*-value that is very close to the limit of 0.05; however, this species shows a significant correlation between the increase in passive search and the number of fasting days with a *P*-value of 0.02. With a *P*-value of 0.06, the passive search for *C. bullatus* also shows a correlation between passive search and fast-

**Table 2.** Summary of the Kendall correlation tests between the different states (‘rest’, ‘passive search’ and ‘active search’) and the fasting days of the different *Conus* species, with *P*-value and associated *R*<sup>2</sup>.

Species	State	<i>P</i> -value	<i>R</i> <sup>2</sup>
<i>C. bullatus</i>	Rest	<b>0.001</b>	0.23194
	Passive search	0.06	0.06309
	Active search	0.81	0.00214
<i>C. catus</i>	Rest	0.059	0.09590
	Passive search	<b>0.02</b>	0.09552
	Active search	0.75	0.00058
<i>C. conco</i>	Rest	<b>0.005</b>	0.16489
	Passive search	0.44	0.00421
	Active search	0.38	0.00285
<i>C. imperialis</i>	Rest	<b>0.004</b>	0.11613
	Passive search	0.14	0.03854
	Active search	0.38	0.01835
<i>C. textile</i>	Rest	0.52	0.00013
	Passive search	0.68	0.00127
	Active search	0.34	0.00666
<i>C. canonicus</i>	Rest	0.79	0.00005
	Passive search	0.11	0.03100
	Active search	0.19	0.03849

Significant *P*-values (<0.05) are shown in bold font.

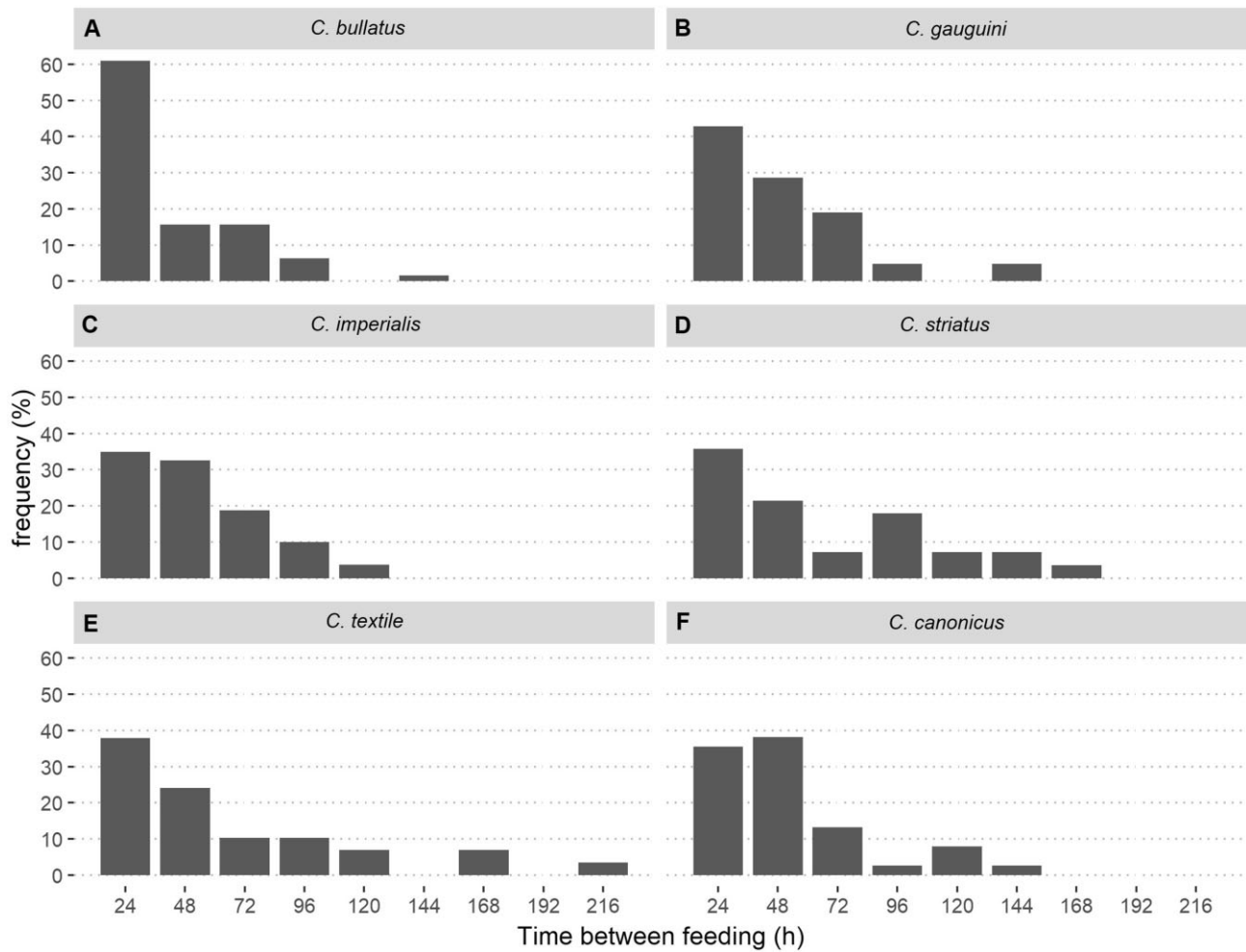


**Figure 4.** Relative prey mass (RPM; prey mass/cone mass × 100) given to different cone species. Two species of molluscivorous cones (*Conus canonicus* and *C. textile*), three species of piscivorous cones (*C. bullatus*, *C. gauguini* and *C. striatus*) and one species of vermivorous cone (*C. imperialis*).

ing time. No significant correlation was found between the different states studied and the number of fasting days for *C. textile* and *C. canonicus*. Correlation values (*R*<sup>2</sup>) were very low, with values ranging from  $5 \times 10^{-5}$  to 0.23. The highest values were those attributed to the linear regression models for rest and for piscivorous and vermivorous species. Despite significant *P*-values, the highest *R*<sup>2</sup> values only varied between 0.09 and 0.23, indicating that fasting only explains a limited part of the activity trends through time.

### *Determining the natural feeding frequency of cone snails*

The relative prey mass used in this experiment varied, on average, between 4.31 and 4.96%, with a mean standard deviation of 0.64%. On average, piscivorous species accepted prey with higher relative mass than species with other feeding regimes (Fig. 4). Some species had many outliers, and in the case of vermivorous species represented by *C. imperialis*, the average was lowered by these outliers. For

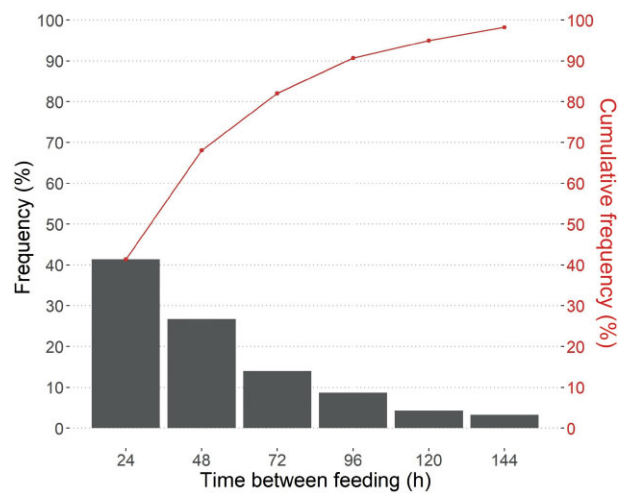


**Figure 5.** Time between feeding: frequency distributions of the different cone snail species **A.** *Conus bullatus*. **B.** *Conus gauguini*. **C.** *Conus imperialis*. **D.** *Conus striatus*. **E.** *Conus textile*. **F.** *Conus canonicus*. The different bar plots represent the percentage of individuals feeding at a certain time interval (% of cones waited  $\times$  hours between feedings).

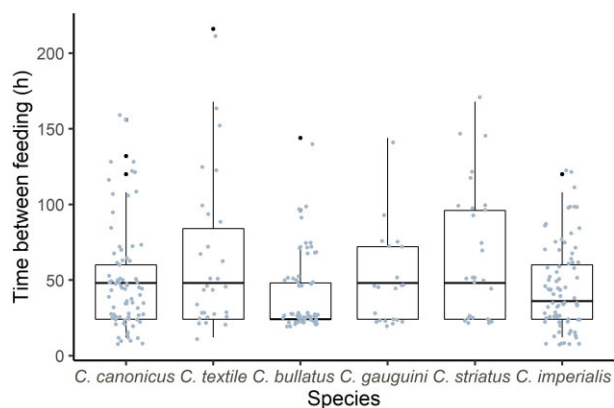
molluscivorous species represented by *C. canonicus* and *C. textile*, the outliers were prey with greater mass, resulting in an increased average. For piscivorous species, the distribution of outliers appeared balanced for *C. bullatus* and *C. striatus*. Overall, these patterns indicate a high individual variance in the food intake rhythm.

Results for the feeding frequency experiment are represented as a bar plot that shows the frequency of individuals as a function of time between two successive feedings (Fig. 5). All species, except *C. bullatus*, presented very similar distributions. The distributions indicate that the time between feedings is predominantly between 24 and 48 h, with a cumulative frequency of over 50% for these two time intervals. The results also indicate that more than 90% of feeding intervals lasted between 24 and 96 h. In the case of *C. bullatus*, however, the frequency exceeded 60% for the 24-h feeding interval, and 90% of feeding intervals lasted between 24 and 72 h. In short, *C. bullatus* required food on a more frequent basis than all other species monitored.

Given the similarities between species in the frequency distribution presented in Figure 5, an average of frequencies was calculated for all the species monitored (Fig. 6). This graph shows that 41% of the cones studied feed, on average, at a frequency of 24 h, followed by 26% for an interval of 48 h between feedings and 14% for an interval of 72 h. With respect to cumulative frequencies, 90% of the



**Figure 6.** Bar plots showing the mean percentage of individuals feeding at a certain time interval. Individuals of *Conus bullatus*, *C. gauguini*, *C. imperialis*, *C. striatus*, *C. textile* and *C. canonicus* were combined in this plot. The curve represents the cumulative frequency.



**Figure 7.** Distribution of time between feeding for *Conus bullatus*, *C. gauquini*, *C. imperialis*, *C. canonicus*, *C. textile* and *C. imperialis*. The Kruskal–Wallis test results:  $\chi^2(5) = 8.63$ ;  $P = 0.12$ ;  $n = 300$ .

individuals fed at an interval between 24 and 96 h and nearly 100% for an interval between 24 and 144 h.

Lastly, we present the time interval between feedings per species with box plots (Fig. 7). The distributions are widespread in some species (*C. canonicus*, *C. textile* and *C. imperialis*), while they indicate clear steps at 24, 48 and 72 h for the other species (*C. bullatus*, *C. gauquini* and *C. striatus*), all three of which are piscivorous. With a  $P$ -value of 0.12, the Kruskal–Wallis test showed no significant difference in time between food intakes between species. Some species fed naturally with an average interval of 40–50 h (*C. bullatus*: 41.6 h; *C. imperialis*: 45.8 h; *C. gauquini*: 49.1 h; and *C. canonicus*: 50.2 h) and others with an average interval of more than 60 h (*C. textile*: 63.3 h; and *C. striatus*: 66 h) (Fig. 7).

## DISCUSSION

The study of the foraging activity of cones during fasting demonstrated a decrease in the time spent at ‘rest’, which then quickly reached a plateau for the vermivorous and piscivorous species. In parallel, the resting state of the molluscivorous species remained stable over the fasting period. The experiment on the natural frequency of feeding yielded distribution curves that showed the frequencies of individuals as a function of the time between two successive feedings and indicated a very strong similarity between all species, with the exception of *Conus bullatus*. When these frequencies were averaged, the overall distribution indicated that 90% of the individuals fed between an interval of 24 and 96 h. Finally, with an average interval of 45–66 h, no significant difference between the feeding frequencies of the species was observed.

### *Investigating the foraging activity to determine a hunger index*

Monitoring foraging activity under food deprivation shows, for most species, a decrease in resting time (i.e. increase in active search) during the first 3–4 days. Defining categories of activity in cones can be challenging since it is unclear what each of the movements may indicate and which may be associated with a search for food. Activity in several species of cones has been studied in the wild for periods spanning several hours (Leviten & Kohn, 1980). In this previous study, cones were defined as active when the shell was lifted by the foot. In our case, we sought to describe cone activity specifically with respect to feeding. We defined three states, including one state with no activity (‘rest’) and two states with the presence of activity (‘passive search’ and ‘active search’). The cone is considered to be in a state of passive search if it moves its siphon, regardless of whether its foot is retracted or extended. This assumption was

based on the fact that the siphon plays an important role in the detection of organic molecules and thus of potential prey (Kohn, 1961). The active search state was defined by a moving shell and therefore a deployed foot, which corresponds to Leviten and Kohn’s definition.

While the active search state was defined to describe a cone’s search for food, the definition is not entirely accurate. It is true that a cone’s movements can be associated with both foraging and the exploration of its environment. However, personal observations have shown that a cone that moves in the aquarium will not necessarily show a predatory behaviour (i.e. extension of the proboscis; Supplementary Material Appendix S1) if a prey is provided. In the end, we arbitrarily defined three states based on activities that were clearly distinct from one another, and we monitored all individuals and all species with the same criteria. This is the first study to show the effect of fasting on nocturnal cone snail activity. In gastropod molluscs, the effect of hunger has already been studied and has been shown to modify behaviour. Notably, in the case of predation, it has been shown that for *Nassarius*, for example, the risk of predation is outweighed by hunger (Morton & Chan, 1999) or that for *Notocochlis tigrina*, hunger will increase the status of predation by drilling (Das, Chattopadhyay & Chattopadhyay, 2015).

In the present study, the main finding is evidence of a decrease in resting time in piscivorous and vermivorous cones, which supports the results of Morton & Chan (1999), who showed that *Nassarius* spp. are more active despite a strong risk of predation. In short, if there is more activity, there is less rest. However, the decrease in resting time was compensated by an increase in passive search time (i.e. olfactory search using the siphon and not by an increase in search by displacement). Several hypotheses can be derived from our result. These include (1) the hypothesis that passive search consumes less energy than active search and is likely favoured in the search for food, and (2) the hypothesis that active search corresponds to exploration and not necessarily to the search for prey, which would explain its stable state. Thus, if movement is not linked to feeding, fasting should have no effect on this activity. (3) A third hypothesis is that active search is in fact a combination of food search and exploration which, during the fasting period, decreases exploration in favour of food search. It is therefore possible that the time allocated to active search remains stable. Aside from these hypotheses, which will require further experimental study, we also must consider the restricted environment of the aquaria used in the experiment. In such a narrow and enclosed environment, olfactory exploration is enough to identify the presence of potential prey without requiring the cones to move to detect them. Molluscs can acquire a lot of information about the place where they live through olfaction (Fischer, 1950), so much so that they may be able to minimize their movements and save energy. As the literature in this field is almost nonexistent, the above hypotheses will have to be tested in future research.

The results also show that the resting time together with the passive search time tends to stabilize after a few days. The onset of this plateau may correspond to hunger in piscivorous and vermivorous cones. However, the absence of a plateau in molluscivorous species does not exclude the absence of hunger, which is potentially expressed in a different way. Noticeably, the molluscivorous species *C. textile* and *C. canonicus* do not show a trend in resting time. The first hypothesis that could explain this is that the quantity of prey provided on the first day of the experiment was not enough to satisfy the hunger in these species. The second hypothesis is that these observations are specific to their mode of feeding. Molluscs are perhaps more accessible than a worm or a fish, which suggests that these molluscivorous species do not need to modify their activity to capture their prey.

Overall, the categories we defined in this study highlight variations in behaviour that are linked to hunger in piscivorous and vermivorous species. The decrease in resting time compensated by the increase in passive search time served as a proxy to evaluate



hunger. This increase reached a plateau, potentially representing the maximum hunger threshold for these cones.

#### *Determining the natural feeding frequency of cone snails*

In this experiment, the objective was to provide prey with an RPM as close as possible to 5%. We observed that the vermivorous cone, *C. imperialis*, had the ability to cut prey and ingest only the desired quantity. Vermivorous cones use a ‘spaghetti-sucking’ approach that consists of sucking up the prey (Bingham, Mitsunaga & Bergeron, 2010). Although there are no data on the subject, this suction seems strong enough to cut a worm. Overall, the results showed that *C. imperialis* cut and ingested a quantity of prey corresponding to 4% of RPM. A similar distribution pattern was observed for most species, with individuals feeding predominantly every 48–72 h. Only *C. bullatus* showed a different distribution, with more than 60% of the individuals feeding every 24 h. Notably, during the manipulations, this species was the only one that did not show signs of fear or hiding but, instead, exhibited signs of aggressiveness. A more active and aggressive behaviour may explain why *C. bullatus* seemed to require a higher quantity of prey. Most species had an average time interval between feedings that ranged from 42 to 66 h. Similar feeding frequencies have been recorded for other gastropod molluscs. The predatory gastropod *Nucella heysseana* (Gul’Bin & Shadrin, 1991) and two species of *Japenthria* (*J. ferrea* and *J. cingulate*) have shown feeding frequencies ranging from 1 to 6 days (Ota & Okeshi, 2000).

This is the first study to investigate and report feeding frequencies in Conidae. The only data currently available in the literature on cone feeding come from two studies, both of which reported feeding their cones once a week (Stewart & Gilly, 2005; Chun *et al.*, 2012). However, neither of these studies investigated the feeding frequency of the Conidae used in their experiments. Our study is therefore the first to specifically investigate the feeding frequency of Polynesian Conidae species. Other studies have demonstrated the effect of feeding frequency in gastropod molluscs, including *Marisa cornuarietis* juveniles, and these have documented a higher growth rate in response to a higher feeding frequency (Selck *et al.*, 2006). It would therefore be interesting to carry out further experiments to determine whether a higher or lower feeding frequency can affect the maintenance of these organisms in the aquarium. Similarly, it would be interesting to repeat the same experiment with different RPMs to explore the transition of this frequency according to the relative mass of prey.

This study provides the first insights into the feeding habits of Conidae in French Polynesia. No study had previously investigated the feeding habits of this genus by looking at hunger and satiety parameters. From these results, we can conclude that most Conidae show a natural feeding frequency of 3 days and signs of hunger by day 5. These results appear consistent in comparison to the natural feeding of other molluscs that have previously shown altered behaviour following fasting as well as a similar feeding frequency. However, many hypotheses associated with these results remain to be tested. Relatively little is known about the many fascinating species found within the family Conidae. However, there is growing interest in the pharmacological richness of their venoms. This is why, in a context of commercial exploitation generated by this interest, it is necessary to continue this research to improve our general knowledge and to develop a long-term management plan for cones in French Polynesia.

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#### SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

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