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Chemical stimuli in coral reefs: how butterflyfishes find their food

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Abstract Animals use sensory stimuli either to assess and select habitats, mates or food, as well as for communication. The present study aimed to understand the behavioural processes enabling several *Chaetodon* species (butterflyfishes) to locate one of their food sources (epibionts present on pearl oyster shells) at Rangiroa atoll (French Polynesia). Among the five species tested, our 2-channel choice flume chamber experiments identified three species that were attracted to their food source by chemical stimuli. HPLC experiments showed that pearl oysters and epibionts have specific and unique chemical fingerprints, either one or nine specific peaks, respectively. Overall, chemical stimuli are emitted by both epibionts (used directly by *Chaetodon auriga, C. lunula* and *C. citrinellus*) and live pearl oysters (used indirectly by *C. auriga* and *C. lunula*) to locate their food source. Biosynthesis of these chemical stimuli could be used to artificially attract butterflyfishes to pearl oyster rearing stations in order to increase the natural cleaning of pearl oyster shells and thus reduce one large cost for this aquaculture.

Keywords Sensory mechanisms · Food cues · Habitat selection · Public information · HPLC

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Introduction

Aquatic animals evolved in a world of dissolved chemicals wherein many animals and plants release literally thousands of small and soluble products that carry information (for review, see Cassier et al. 2000). Animals must distinguish between complex odour signals and respond appropriately to locate mates, food and prey, avoid predators, and select a habitat. This complexity challenges the abilities of animals to extract the necessary information to locate the source of an odour signal (for review, see Derby and Sorensen 2008). There is a wealth of literature on the use of chemosensory cues by aquatic species for

foraging, mate choice, navigation and/or predator avoidance (e.g. Constantino and Salmon 2003; Lecchini and Galzin 2003; Hara 2006; Barata et al. 2009). However, our knowledge of the chemical cues that drive aquatic animal behaviour has rarely been harnessed for mutualistic gain for both the animals and human industry. In this paper, we aim to test the potential of chemosensory cues to be used for an economically important aquaculture resource.

Pearl oyster aquaculture (e.g., *Pinctada margaritifera* in French Polynesia and *Pinctada fucata* in China) is an important economy in the Pacific (De Nys and Ison 2008). Epibionts (i.e. sea-anemones, sponges and ascidians) grow on pearl oyster shells reducing their growth and pearl production, therefore, pearl farmers must "artificially" clean oysters by removing them from the lagoon and removing epibionts using a karcher, a pressure cleaner (Pit and Southgate 2003; De Nys and Ison 2008). This technique is costly and incurs pearl oyster mortality. In French Polynesian Atolls, many pearl farmers noticed that some *Chaetodon* species (butterflyfishes) were attracted toward pearl oysters (De Nys and Ison 2008). Butterflyfishes eat fouling organisms (i.e. epibionts) and thus clean pearl oyster shells naturally. This "natural" cleaning is less costly and results in lower mortality of pearl oysters in comparison with "artificial" cleaning. Therefore, identifying the chemical stimuli used by butterflyfishes to locate pearl oysters could be beneficial to the industry (Southgate and Lucas 2008). Our study aims to provide new information about chemosensory cues used by butterflyfishes in order to regulate the biology of aquaculture systems. The abilities of five species of butterflyfishes to locate pearl oysters via chemical stimuli were investigated, under the broader goal of providing recommendations for a natural cleaning method for oyster bed aquaculture.

In the present study, we examined the chemical stimuli used by five *Chaetodon* species to locate pearl oysters (*P. margaritifera*). Among the five species tested at Rangiroa atoll (French Polynesia), four were generally observed on pearl oyster rearing stations (*Chaetodon auriga, C. lunula, C. citrinellus* and *C. ulietensis*) and we also chose one species never observed on the rearing stations (*C. ephippium)*. Specifically, we conducted 1) a series of aquaria experiments to verify the chemical abilities of butterflyfishes to detect pearl oysters and/or epibionts, and 2) biochemical experiments to highlight the chemical fingerprints of pearl oysters and epibionts.

Material and methods

Study location

This study was conducted on the North coast of Rangiroa lagoon, French Polynesia (14°57′48 S, 147° 38′79 W) in October 2007 and April 2008. Forty adults of each *Chaetodon* species (*C. auriga, C. lunula, C. citrinellus, C. ulietensis* and *C. ephippium*) were captured with hand nets. Captured fish were maintained, for 24 h prior to experiments, in aquaria (1.0×1.0×0.8 m) supplied with flow-through sea water from the adjacent lagoon, and without any added artificial or natural habitats.

Experiment 1: Chemical preferences of *Chaetodon* species

The response of *Chaetodon* species to olfactory cues of live pearl oysters *vs*. epibionts was tested in a 2-channel choice flume (70×30 cm, with a water depth of 10 cm), as described by Gerlach et al. (2007). Fish were released at the downstream end of the flume where they were free to move to either side of the chamber and swim upstream towards the preferred water source (Fig. 1). Two tanks were connected to the choice flume by pipes to create a

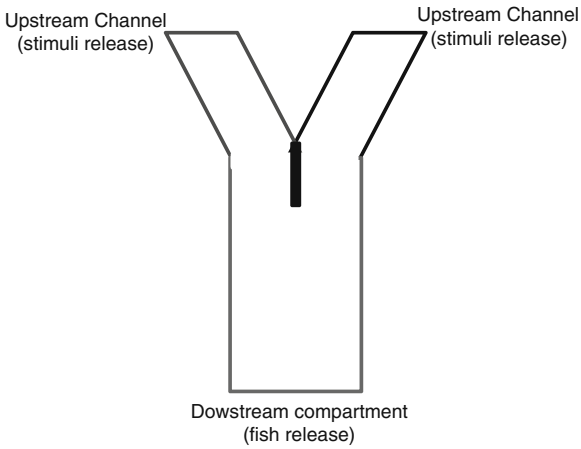

Fig. 1 Experimental 2-channel choice flume used to evaluate chemical cues used by five *Chaetodon* species to locate pearl oysters (*P. margaritifera*)

constant gravity-driven flow (3.33 L.min^{-1} per upstream channel).

For each trial (see next paragraph for more explanation about the different trials), a single fish was placed in the centre of the downstream end of the choice flume (downstream compartment) for a 1 min acclimatisation period (with a net prohibiting the fish to move into the upstream channels). At the end of the acclimatisation period, the net was removed, and then the position of the fish either in the two upstream channels (A or B) or in the downstream compartment was recorded every second. The trial was finished after a 3-min period. The observer was 3 m from the tank and always in the same fixed position, therefore, treatments were randomly placed relative to the observer. This sampling strategy has been validated by previous studies (Lecchini et al. 2007, 2010).

Using the protocol described above, four tests were conducted with ten fish of each species per test. Test 1: We first constructed an expected distribution of directional movement in the absence of manipulated cues with flow-through sea water from the adjacent lagoon in the two tanks connected to the channel choice flume ("control" for tank artifacts). We then determined the choice preference exhibited by a *Chaetodon* presented with: Test 2: water from live pearl oysters in one channel (five epibiont-free pearl oysters which had been immersed in 15 l for 3 h) *vs*. water from the adjacent lagoon in the other channel; Test 3: water from epibionts in one channel (epibionts collected from 5 live pearl oysters which had been immersed in 15 l for 3 h) *vs.* water from the adjacent lagoon in the other channel; and Test 4: water from live pearl oysters in one channel *vs*. water from epibionts in the other channel. After each trial (ten trials / species / test), the flume chamber and the two tanks were emptied and washed with freshwater. The position of the channel containing each water type (live pearl oysters, epibionts or lagoon water) was randomly alternated after each trial.

For each *Chaetodon* species a Student *t*-test was firstly carried out to compare the time spent in the downstream compartment in the control test *vs.* in the other tests (test 2, 3 or 4) in order to show that the downstream time differed from baseline (i.e. fish decrease the time spent in the downstream compartment when the upstream channel was running with water from live pearl oysters or epibionts—tests 2, 3 and 4). Secondly, a Student *t*-test was carried out to compare the time spent in each of the two upstream channels in each test (tests 2, 3 and 4) in order to determine fish preferences

Experiment 2: Chemical fingerprints of pearl oysters and epibionts

High Performance Liquid Chromatography (HPLC) was used to determine the chemical fingerprints of pearl oysters and epibionts. We collected (1) 15 l of water from tanks containing five epibiont-free pearl oysters which had been immersed for 3 h, (2) 15 l of water from tanks containing epibionts collected from five live pearl oysters which had been immersed for 3 h, and (3) 15 l of water from tanks supplied from the adjacent lagoon.

The three different seawater collections were filtered under vacuum through Solid Phase Extraction (SPE) cartridges containing a C_{18} silica-gel based bonded phase sorbent, then washed with 50 ml of distilled water and then desorbed with 50 ml of methanol. The organic phase of each collection was then freeze-dried leaving a powdery organic residue. The organic extracts from the three water samples were dissolved in 1 ml of methanol before analysis. HPLC was performed with a system from Waters including the Alliance separations module 2,695, the column heater and the 2,998 photodiode array detector. The liquid chromatograph was equipped with a 5 μm Phenomenex GeminiC18 column (150×3.00 mm). The equipment was controlled and the data were handled using Empower Chromatography Data software (Waters). The following analytical solvent and gradient systems were used: A, 0.1% aq. trifluoroaceticacid; B, acetonitril:trifluoroaceticacid (99.9:0.1, v:v); linear gradient from 25% B to 100% B within 15 min and then 100% B in additional 5 min; the flow rate was 0.5 ml.min^{-1} and injection volume was 20 μl. Column temperature was set at 30°C, and the data collection across the 210–500 nm wavelength range was performed as follows: sampling one point per second, resolution 1.2 nm and no smoothing. To maximize sensitivity, the data were processed to create a Max Plot chromatogram which plots the maximum spectral absorbance measured at each time point. The Max Plot enables the detection of all detectable UV-absorbing components in the sample.

Results

Experiment 1: Chemical preferences of *Chaetodon* species

In the absence of manipulated cues (baseline distribution—test 1), the ten individuals of each species stayed mainly in the downstream compartment (Fig. 2a). When one channel was filled with water from live pearl oysters (test 2—Fig. 2b), two *Chaetodon* species had a distribution that differed from the baseline distribution (first Student *t*-test: *t*-value=28.8, $P<0.001$ for *C. auriga;* *t*-value=26.5, $P=0.001$ for *C. lunula*); live pearl oysters were preferred over lagoon water (second Student *t*-test: *t*-value=15.1, $P=0.01$ for *C. auriga;* *t*-value=17.2, $P=0.009$ for *C. lunula*). For the other three species, fish did not show any preference for water from live pearl oysters (Student *t*-tests: $P>0.05$).

In epibionts water *vs*. lagoon water test (test 3—Fig. 2c), three *Chaetodon* species had a distribution that differed from the baseline distribution (first Student *t*-test: *t*-value=19.5, $P=0.007$ for *C. auriga;* *t*-value=27.1, $P<0.001$ for *C. lunula;* *t*-value=14.7, $P=0.01$ for *C. citrinellus*); epibionts were preferred over lagoon water (second Student *t*-test: *t*-value=28.3, $P<0.001$ for *C. auriga;* *t*-value=27.3, $P<0.001$ for *C. lunula;* *t*-value=21.1, $P=0.004$ for *C. citrinellus*). For the other two species, fish did not show any preference for water from epibionts (Student *t*-tests: $P>0.05$).

In live pearl oysters water *vs*. epibionts water test (test 4—Fig. 2d), three *Chaetodon* species had a distribution that differed from the baseline distribution (first Student *t*-test: *t*-value=27.9, $P<0.001$ for *C. auriga;* *t*-value=19.1, $P=0.008$ for *C. lunula;* *t*-value=11.7, $P=0.02$ for *C. citrinellus*). Live pearl oysters were preferred over epibionts for *C. lunula* (second Student *t*-test: *t*-value=14.3, $P=0.01$). In contrast, epibionts were preferred over live pearl oysters for *C. auriga* (second Student *t*-test: *t*-value=22.8, $P=0.003$) and for *C. citrinellus* (second Student *t*-test: *t*-value=18.3, $P=0.008$). For the other two species, fish did not show any preference for water from live pearl oysters or epibionts (Student *t*-tests: $P>0.05$).

Experiment 2: Chemical fingerprints of pearl oysters and epibionts

HPLC analysis showed that the four major peaks on the chromatogram of lagoon water were also present on the chromatograms of live pearl oysters (except one peak) and epibionts (shown by arrows in Fig. 3). We interpreted these shared patterns as background signatures in seawater (pearl oysters and epibionts were immerged in lagoon water). However, the chromatogram of pearl oysters showed one major peak that was not present in lagoon water (shown by a black star in Fig. 3). Furthermore, the chromatogram of epibionts showed a higher molecular diversity than the chromatogram of lagoon water with nine major peaks specific to the epibiont chromatogram (shown by black circles in Fig. 3). We interpreted these different patterns as pearl oysters and epibionts having specific and unique chemical fingerprints.

Discussion

In the present study, we tested the hypothesis that butterflyfishes use chemosensory cues to locate potential foraging sites. Our 2-channel choice flume chamber experiments showed that *C. ephippium,* the species never observed on pearl oyster rearing stations, was neither attracted by odours from live pearl oysters nor epibionts (Fig. 2). Among the four species generally observed on rearing stations (*C. auriga, C. lunula, C. citrinellus* and *C. ulietensis*), our experiments identified three species that were attracted to chemical stimuli of their food source: chemical stimuli are either emitted by epibionts themselves (direct public information from food used by *C. auriga, C. lunula* and *C. citrinellus*) or by live pearl oysters (indirect public information used by *C. auriga* and *C. lunula*).

Our results confirm field observations as *C. auriga, C. lunula* and *C. citrinellus* are found on pearl oyster rearing stations at Rangiroa (Che et al. 1996). However, *C. ulietensis* is also observed on rearing stations, but our experiments showed that this species was neither attracted by odours from live pearl oysters nor from epibionts. *Chaetodon ulietensis* may therefore be able to detect the presence of pearl oyster rearing stations by other sensory cues, possibly indirectly using cues from other *Chaetodon* species while themselves feeding on epibionts at the rearing stations. This tendency to follow a conspecific or an individual from another species to a food location (i.e. social learning) has been shown in some mammals (Reader et al. 2003; Kendal et al. 2009), but not fishes, although fishes are known to aggregate at food

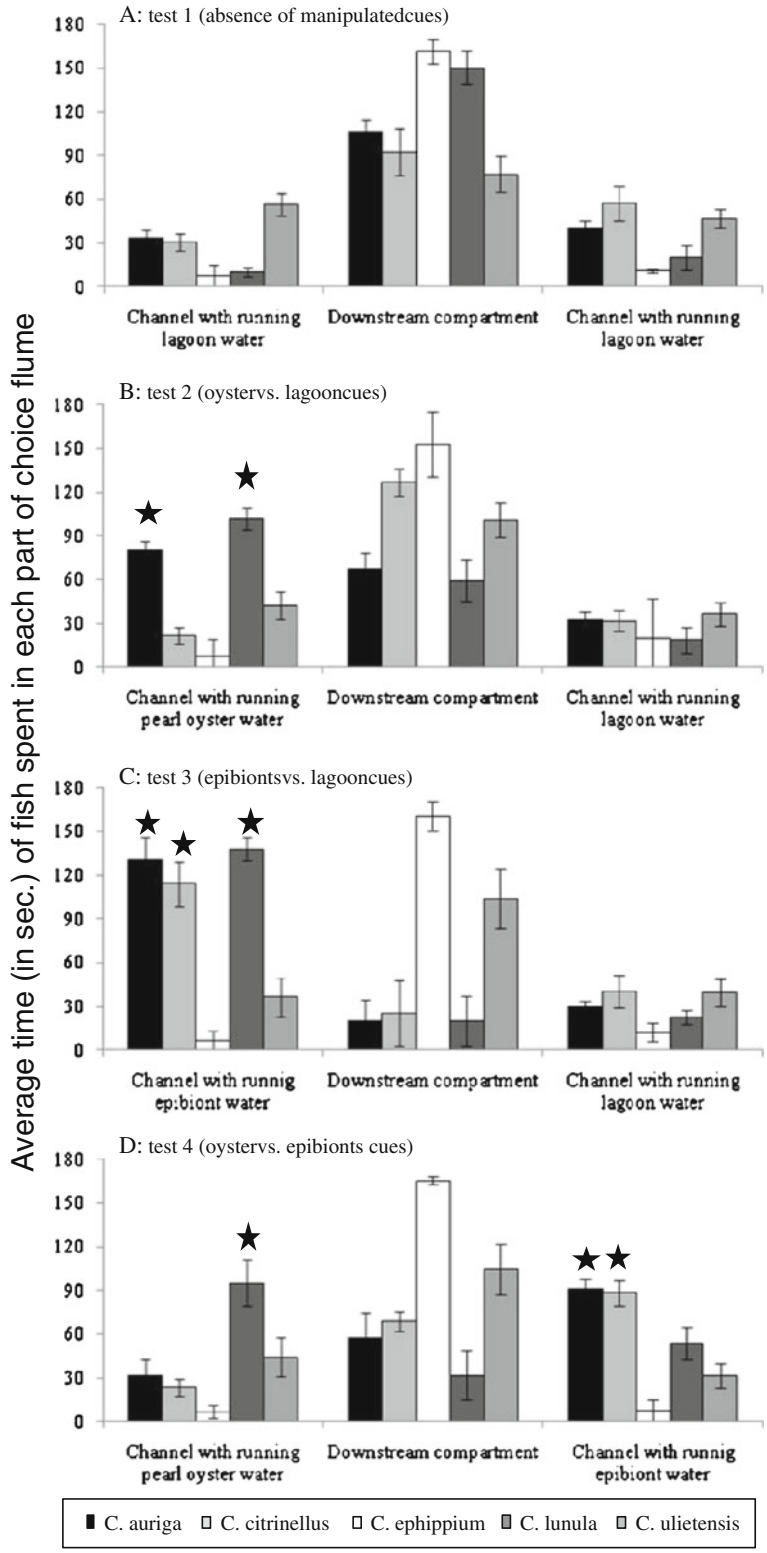

Fig. 2 Chemosensory preferences of five *Chaetodon* species (*C. auriga, C. citrinellus, C. ephippium, C. lunula* and *C. ulietensis*) in four tests carried out in a 2-channel choice flume chamber. Mean (± 1SE) time spent in each chamber of the choice flume in four tests: Fish were presented **a** with water in the absence of manipulated cues (test 1), **b** with water from live pearl oysters *vs*. water from the adjacent lagoon (test 2); **c** with water from epibionts *vs*. water from the adjacent lagoon (test 3); and **d** with water from live pearl oysters *vs*. water from epibionts (test 4). The black star represents a significant attraction of fish toward water from live pearl oysters or epibionts

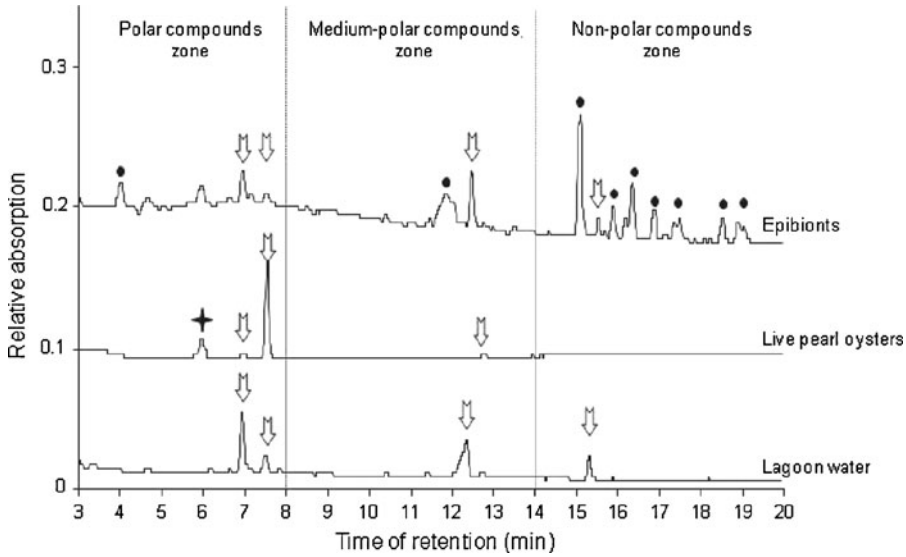

Fig. 3 HPLC chromatograms of seawater collected from tanks containing pearl oysters or epibionts, or from tanks supplied with water from the adjacent lagoon (lagoon water). We deleted the first 3 min of chromatograms as it corresponds to dead volume of the column. The chromatograms were divided into three zones (Mant and Hodges 1991): one zone where polar compounds were eluted (3–8 min), one zone where medium-polar compounds were eluted (8–14 min) and one zone where non-polar compounds were eluted (14–20 min). To maximize sensitivity, the data were processed to create Max Plot chromatograms which plots the maximum spectral absorbance measured at each time point. *Arrows* represent the major peaks in common with all water samples, but with varying degrees of absorption quantity. The *black star* represents the major peak specific to the pearl oyster chromatogram. *Black circles* represent the major peaks specific to the epibionts chromatogram

sources. Overall, our study highlights that public information is used by butterflyfishes to find their food source (epibionts present on pearl oysters): either directly from the food source or indirectly from organisms associated with the food source. Moreover, HPLC experiments showed that pearl oysters and epibionts had specific and unique chemical fingerprints (Fig. 3). Identifying the nature of these chemical cues used by butterflyfishes to detect the presence of pearl oyster rearing stations would be beneficial for pearl oyster aquaculture. Although some *Chaetodon* species (*C. auriga, C. lunula, C. citrinellus* and *C. ulietensis*) were observed cleaning on rearing stations, their presence is random and often in low abundance. However, rearing stations should be cleaned on a monthly basis to minimise fouling (Che et al. 1996; Southgate and Lucas 2008). Therefore, the biosynthesis of a chemical stimulus could be used to artificially attract butterflyfishes to rearing stations, and increase the natural cleaning of pearl oyster shells.

In future studies at Rangiroa, our objective will be to identify the peak(s) responsible for *Chaetodon* attraction (i.e. to test the attractive power of firstly the major peak in the pearl oyster fingerprint and secondly the nine major peaks of the epibiont fingerprint). Once the peak (s) responsible for *Chaetodon* attraction have been highlighted, additional biochemical analyses (e.g., mass spectrometry coupled with liquid chromatography) will be conducted to identify the structural composition of the organic compound. The final objective will be to add odour diffusers to artificial habitats near to pearl oyster stations in order to artificially attract butterflyfishes and increase natural cleaning.

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