Insight into biting diversity to capture benthic prey in damselfishes (Pomacentridae)

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A B S T R A C T

The cerato-mandibular (c-md) ligament, joining the hyoid bar to the coronoid process of the angular, allows Pomacentridae to slam their mouth shut in a few milliseconds. Previous works have revealed that such a mechanism is used to feed, but some variability in biting patterns has been observed between two damselfish species. The pelagic feeder *Amphiprion clarkii* performs two different kinematic patterns to bite fixed prey, one that does not depend on the c-md ligament (*biting-1*) and one that does (*biting-2*). The benthic feeder *Stegastes rectifraenum* only performs *biting-2*. The present study aims to shed light on the occurrence of *biting-2* in the feeding behaviour of Pomacentridae. To test our hypothesis that *biting-2* would be the only biting pattern for benthic feeders, we compared biting behaviours among four species: one pelagic feeder, *A. clarkii*, and three benthic feeders, *Neoglyphidodon nigroris*, *Stegastes leucostictus* and *S. rectifraenum*. Our results showed that the four species were able to perform *biting-2*, but they do not support that the use of this pattern is related to trophic habits. Contrary to *S. rectifraenum*, the two other benthic feeders randomly used *biting-1* and *biting-2* patterns, similar to *A. clarkii*. Two hypotheses are discussed for explaining this variability within Pomacentridae. Finally, it has been recently shown that some damselfishes do not possess the c-md ligament. We therefore included two species lacking the c-md ligament (*Chromis chromis* and *Abudefduf troschelii*) in our study and we demonstrate our expectation that they are unable to perform *biting-2*.

1. Introduction

A vast majority of ray-finned fishes use suction to capture elusive or loosely attached prey (Case et al., 2008; Ferry-Graham et al., 2001; Norton and Brainerd, 1993; Oufiero et al., 2012; Van Wassenbergh and De Rechter, 2011; Wainwright et al., 2007, 2001; Waltzek and Wainwright, 2003). Despite the diversity of fishes using this feeding mode, the movements leading to suction are highly conserved in teleosts (Lauder, 1985, 1982; Sanford and Wainwright, 2002; Westneat and Wainwright, 1989). Conversely, the less common biting mode used to dislodge firmly attached prey seems highly diversified. Some taxa, such as Labridae, rely on a strong suction force used in combination with biting (Ferry-Graham et al., 2002). Other taxa rely more on their biting performance and often have a morphological novelty facilitating such a biting mode. The Scarinae are characterised by their fused beak-like jaws used to scrape or excavate algae and/or live coral from substrata (Bellwood and Choat, 1990). The Pomacanthidae are

able to occlude their oral jaws while they are still protruded thanks to an intramandibular joint between the angular and the dentary of the lower jaw. This functional novelty allows angelfish to adopt their particular "grab-and-tear" feeding behaviour to feed on cryptobenthic resources (Konow and Bellwood, 2005). In Acanthuridae, Girellidae, Poeciliidae and some Scarinae, an intramandibular joint has the opposite function since it allows a gape expansion to scrape a larger surface of the substratum (Ferry et al., 2012; Konow et al., 2008; Purcell and Bellwood, 1993; Streelman et al., 2002; Vial and Ojeda, 1990).

Recently, a new biting mechanism has been highlighted in Pomacentridae (damselfishes) (Olivier et al., 2015, 2014; Parmentier et al., 2007). This mechanism relies on the cerato-mandibular (c-md) ligament, joining the ceratohyal of the hyoid bar to the coronoid process of the angular of the lower jaw (Fig. 1). Manipulations of freshly euthanised fish and the use of high-speed video recording of live fish feeding with X-ray and visible light sources allowed us to determine how this mechanism works (Olivier et al., 2015, 2014; Parmentier et al., 2007). When the neuro-cranium and the hyoid apparatus are held at rest, the c-md ligament is loose and cannot transmit movement to the lower jaw (Fig. 2a). Pulling along the line of action of the epaxial muscles rotates the
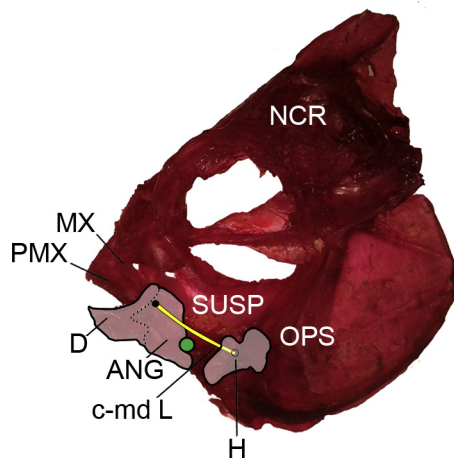
Fig. 1. Left lateral view of a *Neoglyphidodon nigroris* skull on a cleared and stained specimen. The left oral jaws, suspensorium, opercles, and hyoid bar have been removed allowing a view of the right part of the hyoid apparatus in the buccal cavity. The lower jaw and hyoid bar are highlighted. The green dot represents the quadrate articulation. The cerato-mandibular (c-md) ligament, highlighted in yellow, inserts on the inner (medial) part of the coronoid process of the angular and on the external (lateral) face of the ceratohyal of the hyoid bar. ANG: angular, c-md L: cerato-mandibular ligament, D: dentary, H: hyoid bar, MX: maxillary, NCR: neurocranium, PMX: premaxillary, OPS: opercular series, SUSP: suspensorium. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

neurocranium clockwise (left-facing lateral view). This rotation, via a four-bar linkage (see Muller et al., 1982 for details), causes the hyoid bar to rotate counterclockwise which induces mouth opening and gradually moves the insertion points of the c-md ligament away, until it is fully tightened (Fig. 2b). At this stage, additional elevation of the neurocranium and/or depression of the hyoid bar make(s) the taut ligament close the mouth by inducing rotation of the lower jaw around its quadrate articulation (Fig. 2c). High-speed video analyses revealed that the movements of the neurocranium and the hyoid apparatus are fast, which quickly tightens the ligament, ultimately allowing the fish to slam their mouth shut within 2–4 ms (Olivier et al., 2015, 2014; Parmentier et al., 2007).

It was first highlighted that the "mechanism involving the cerato-mandibular ligament" (hereafter, c-md mechanism) is used during acoustic communication in damselfishes, the sound being produced by an oral-teeth collision (Colleye and Parmentier, 2012; Parmentier et al., 2007). It was later illustrated that the same mechanism is also used during feeding (Olivier et al., 2015, 2014). There are three main trophic guilds in damselfishes: (1) the pelagic feeders that feed mainly on planktonic copepods, (2) the benthic feeders that mainly graze filamentous algae and (3) an intermediate group including species that forage for their prey in the pelagic and the benthic environments in variable proportions (*e.g.* planktonic and benthic copepods, small vagile invertebrates and filamentous algae) (Frédérich et al., 2016, 2009). A study on a zooplanktivorous species, *Amphiprion clarkii* (Bennett, 1830), showed that the c-md mechanism is never used to capture elusive prey (Olivier et al., 2015). However, *A. clarkii* performs two different kinematic patterns to seize fixed prey items, which are called *biting-1* (*B1*) and *biting-2* (*B2*). *Biting-1* does not look like the kinematic pattern for sound production and *B1* is not dependent on the c-md ligament because the same kinematic pattern can be performed after its surgical transection; the fish uses the *adductor mandibulae* (AM) to close its mouth (Olivier et al., 2015). Conversely, *B2* shows the same kinematic pattern as sound production and cannot be performed after the c-md ligament transection (Olivier et al., 2015). This study highlighted that the c-md mechanism is used in two different behaviours, *i.e.* to feed and to communicate. However, the
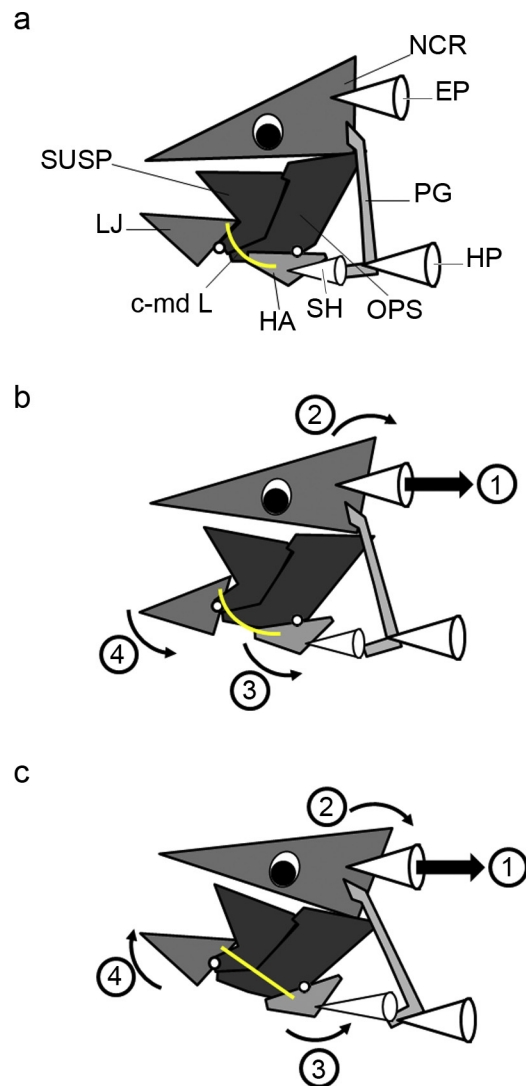

Fig. 2. Illustration of the cerato-mandibular ligament mechanism. As in Fig. 1, the left oral jaws, suspensorium, opercles, and hyoid bar have been removed to allow a view of right part of the hyoid apparatus in the buccal cavity. White cones indicate three muscles: the epaxial muscles (EP), hypaxial muscles (HP) and *sternohyoideus* muscle (SH). White circles indicate two articulations, one between the lower jaw and the suspensorium and another between the hyoid apparatus and the opercular series. c-md L: cerato-mandibular ligament, HA: hyoid apparatus, LJ: lower jaw, NCR: neurocranium, PG: pectoral girdle, OPS: opercular series, SUSP: suspensorium. In (a) no movement occurs and the c-md ligament is loose. In (b) a contraction on the epaxial muscle (1) induces a rotation of the neurocranium (2) provoking a rotation of the hyoid apparatus (3) which results in mouth opening (4) and in the tightening of the c- md ligament. In (c) additional contraction of the epaxial muscles (1) induces greater rotations of the neurocranium and hyoid apparatus (2 and 3) which results, as the c-md ligament is now fully tightened, in mouth closing (4). The same phenomenon of both mouth opening and closing can be obtained by pulling along the line of action of the *sternohyoideus*/hypaxial muscles but there is no neurocranium elevation in this case.

reasons for using two different biting patterns (*B1* and *B2*) to seize fixed prey remained unanswered. A different outcome has been observed in another damselfish, the grazer *Stegastes rectifraenum* (Gill, 1862) (Olivier et al., 2014). Instead of using two different patterns to seize fixed food items, this species only performs a *B2*-like kinematic pattern; a *B1*-like pattern being only performed after transection of the c-md ligament (Olivier et al., 2014). Such variability in the use of biting patterns between damselfish species is still unexplained. To better understand the diversity of biting in the feeding behaviours of Pomacentridae, we aim to test whether the use of one or two biting patterns could be related to trophic

habits. We expect that *B2* is an essential pattern for benthic feeders. To test this hypothesis, we compare the biting patterns used by various benthic feeders from different subfamilies (Stegastinae and Pomacentrinae). Recent findings also showed that some planktivorous damselfishes lack the c-md ligament (Frédérich et al., 2014). Therefore, we also aim to check our hypothesis that these species without the c-md ligament are unable to perform a *B2* pattern.

2. Material and methods

2.1. Specimens and husbandry

Six species were studied. Four of these have the c-md ligament: *Neoglyphidodon nigroris* (Cuvier and Valenciennes, 1830), *Stegastes leucostictus* (Schomburgk, 1848) and *S. rectifraenum* are benthic feeders (Ceccarelli, 2007; Emery, 1973; Kuo and Shao, 1991; Montgomery, 1980; Randall, 1967); *Amphiprion clarkii* is a pelagic feeder (Kuo and Shao, 1991). The two species lacking the c-md ligament, *Abudefduf troschelii* (Gill, 1862) and *Chromis chromis* (Linnaeus, 1758), are pelagic feeders (Aguilar-Medrano et al., 2011; Dulčić, 2007). Individuals of *S. rectifraenum*, *A. troschelii* and *C. chromis* were caught in the wild using clove oil and hand nets. The first two were studied at La Paz in the Gulf of California (Mexico) at the "Centro de Investigaciones Biológicas del Noroeste (CIBNOR)". *Chromis chromis* was studied in Corsica (France) at the "Station de Recherches Sous-marines et Océanographiques (STARESO)". Individuals of *A. clarkii*, *N. nigroris* and *S. leucostictus* came from the marine ornamental trade. The three species caught in the wild were kept in flow-through tanks (750 l in Baja California, 300 l in Corsica) at ambient temperature (24–26 °C in Baja California and around 23 °C in Corsica), and subject to natural photoperiod (14L:10D for both places). Species from shops were kept in tanks (300 l) in the laboratory at Liège (Belgium) at a temperature of 25 °C with a 12L:12D photoperiod. The six species belong to the four main subfamilies of the Pomacentridae and represent the trophic diversity of the family (Table 1).

Experimental and animal care protocols followed all relevant international guidelines and were approved by the ethics commission of the University of Liège (protocol no. 113). Outside experimental periods, fishes were fed three times a week with diverse food items such as pellets, nauplii of *Artemia*, pieces of mussel/shrimp and/or filamentous algae on small rocks sampled directly in the field.

2.2. High-speed video recordings

To study the kinematics of feeding behaviours, a high-speed video camera (RedLake MotionPro 2000, San Diego, USA; 1024 × 1280 pixels and Model NX4-S1, IDT, Tallahassee, USA; 640 × 456 pixels) was used to record movements at 500 frames per second (fps). Specimens were placed in individual tanks (∼100 l) in which a wall was used to create a 10 cm width corridor provided with a shelter. This narrow space facilitates the acquisition of analysable video recordings in which the fish is in lateral view while feeding. For all six species, we studied biting on food items fixed to tweezers (pieces of shrimp or mussel of roughly 1 cm long and 0.5 cm wide). The data for *A. clarkii* and *S. rectifraenum* come from our previous works (Olivier et al., 2015, 2014).

2.3. Transection of the cerato-mandibular ligament

The role of the c-md ligament in feeding was experimentally tested by its surgical transection. Movements were compared before and after the section. For the surgical operation, fish were first anesthetised with 200 ppm of tricaine methanesulfonate (MS-222). The individuals were then operated on using a dissecting

Table 1
Number of video sequences per species, behaviours and individuals.

Amphiprion clarkii (Pelagic feeder; Pomacentrinae)							
Individuals	1	2	3	4	5	6	7	Total
SL (cm)	8.4	4.9	5.2	5.7	5.3	6.5	5.9	
B1	5	4	3	2	–	2	–	16
B2	–	–	2	3	4	–	4	13
B*	–	–	–	6	–	4	3	13

Stegastes rectifraenum (Benthic feeder; Stegastinae)							
Individuals	1	2	3	4	5	6	
SL (cm)	9.7	10	10.1	10	10.1	9.9	
B2	5	5	5	–	–	–	15
B*	5	5	5	–	–	–	15

Neoglyphidodon nigroris (Benthic feeder; Pomacentrinae)			
Individuals	1	2	3	
SL (cm)	6.3	8.1	7.6	
B1	3	2	–	5
B2	4	1	4	9
B*	–	13	2	15

Stegastes leucostictus (Benthic feeder; Stegastinae)			
Individuals	1	2	3	
SL (cm)	6.2	5.5	5.3	
B1	5	4	5	14
B2	–	–	1	1
B*	3	7	–	10

Abudefduf troschelii (Pelagic feeder; Abudefdufinae)		
Individuals	1	2	
SL (cm)	6.9	6.2	
BX	8	7	15

Chromis chromis (Pelagic feeder; Chrominae)			
Individuals	1	2	3	
SL (cm)	6.5	8	8.7	
BX	8	2	5	15
Total				**156**

B1: biting-1; *B2*: biting-2; *B**: biting after the transection of the cerato-mandibular (c-md) ligament; *BX*: biting when the c-md is naturally absent. SL: standard length.

microscope by inserting a thin blade between the lower jaw and the hyoid bar to cut the c-md ligament. This operation was performed on both right and left sides. Disinfectant (containing Propolis Alcohol) was then injected into the buccal cavity. Because previous examinations have shown that an intact c-md ligament only allows the closing of the mouth when fish are manipulated by pulling backward on the epaxial or the *sternohyoideus* muscles, it was possible to check the success of the surgery with these manipulations. The fish that were successfully operated were then placed in a recovery bucket with oxygenated seawater. Once the fish started to move again, they were placed in their individual corridor and their recovery was monitored for 30 min. The animals were then left at rest for a period of 24 h. Then, individuals were presented with food and their feeding behaviour was recorded with the high-speed video camera. At the end of the experiment, all fish were euthanised and dissected to check the section of the c-md ligament on left and right sides and to ensure that other structures were not damaged.

2.4. Video analyses

We analysed only the video sequences with fish in lateral view with all the points of interest clearly visible. We attempted to collect at least three capture events for three individuals of each species (Table 1). However, *A. troschelii* was very difficult to capture in the wild and only two individuals were used. One individual of *N. nigroris* died before the transection experiment, so only two individuals were studied after the transection of the c-md ligament in this species. We had to obtain analysable videos quite rapidly following the c-md ligament transection because it can regenerate. After several attempts, we did not manage to obtain usable movies for indivual-3 of *S. leucostictus*.

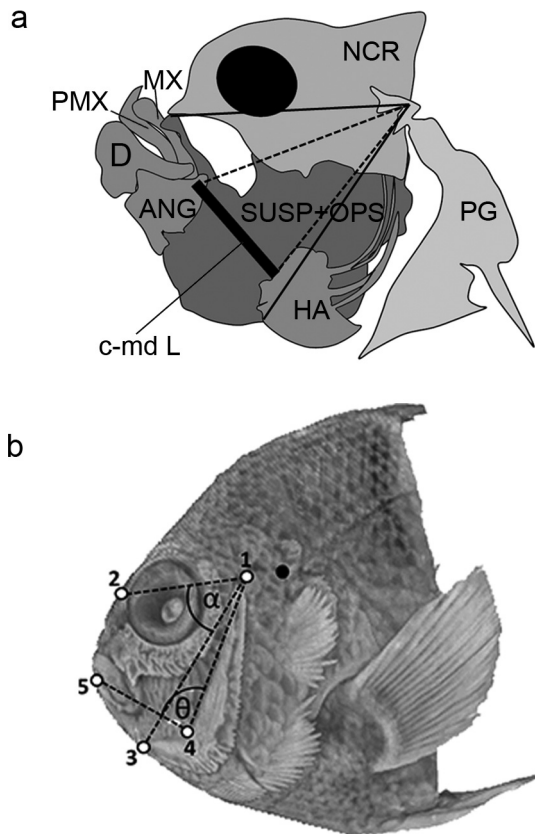**Fig. 3.** (a) Schematic illustration of the skull of *Amphiprion clarkii*. The left oral jaws, suspensorium, opercles and hyoid bar have been removed to allow a view of right part of the hyoid apparatus in the buccal cavity. The angle measuring the relative movement between the two insertion points of the c-md ligament is represented with dotted lines. The angle measuring the relative movement between the neurocranium and the hyoid apparatus is represented with solid lines. ANG: angular, c-md L: cerato-mandibular ligament, D: dentary, MX: maxillary, NCR: neurocranium, OPS: opercular series, PG: pectoral girdle, PMX: premaxillary, SUSP: suspensorium. (b) Illustration of the digitised variables. 1: hyomandibular articulation; 2: neurocranium; 3: hyoid apparatus; 4: rostral corner of the preopercular; 5: dentary of the lower jaw. The angle α measures the relative distance between the neurocranium and the protrusion of the hyoid apparatus, the angle θ measures the lower jaw rotation. The black landmark indicates the articulation between the neurocranium and pectoral girdle. This figure has been modified from Olivier et al. (2015).

2.5. Selection of the variables measured

The selection of the variables was based on previous studies, these variables clearly allow the distinction between *B1*-like and *B2*-like patterns (Olivier et al., 2015, 2014). Although we cannot directly measure the movement between the c-md insertion points (angle between dotted lines on Fig. 3a) from an external view, we were able to monitor movements of the structures causing the movement of these elements i.e. the neurocranium and the hyoid apparatus. Previous studies showed the angle measuring the relative movement between the anterior part of the neurocranium and the hyoid apparatus (angle between solid lines on Fig. 3a) adequately reflected the movement between the insertion points of the c-md ligament (Olivier et al., 2015, 2014). Images were digitised frame by frame using MIDAS 2.0 and Motion Studio 64 softwares. Five landmarks were chosen (Fig. 3b) to quantify cranial movements during biting behaviour. The five landmarks were: (1) hyomandibular articulation on the neurocranium; (2) a point on the anterior part of the neurocranium (on the edge of the neurocranium in front of the most anterior part of the eye); (3) a point on the anterior part of the hyoid apparatus (urohyal) (easily visible in Pomacentridae because of the absence of the *hyohyoideus*

muscle); (4) the rostral corner of the preopercular and (5) the anterior tip of the dentary (lower jaw). The hyomandibular articulation (landmark 1) is close to the joint between the post-temporal and supracleithrum bones of the pectoral girdle, which is the pivot point of the neurocranium (Fig. 3b). These points allowed the calculation of seven kinematic variables including maximum angles, angular speed, angular acceleration, and the duration of kinematic events which were used to describe the different kinematic patterns. The variables measured were (1) the maximum relative movement between the neurocranium and the hyoid apparatus (MRM) which was measured by the angle α (°) (Fig. 3b); (2) the maximal lower jaw depression which was measured by the angle θ (°) (Fig. 3b); (3) the mean angular closing speed (°s^{-1}) of the lower jaw; (4) the peak instantaneous angular acceleration (°s^{-2}) by which the neurocranium and the hyoid apparatus moved away from one another; (5) the duration (ms) between the time of the bite and the MRM; (6) the synchronisation (ms) between hyoid apparatus and lower jaw displacements, measured as the time between maximal displacement of the hyoid apparatus and the maximal displacement of lower jaw elevation and (7) the mouth closing duration (ms). Each analysis started with the onset of mouth opening and finished at the seizure of fixed food items.

2.6. Statistical analysis of the kinematic data

We first performed a principal component analysis (PCA, using the correlation matrix Statistica, version 10) with the seven kinematic variables to have an exploratory view of our dataset. The PCA results explored the biting diversity prior to any classification. Then, according to the PCA results, we classified the kinematic patterns as *B1* or *B2*. In addition to *B1* and *B2* classification, the bites performed after the c-md ligament transection were termed *B**; the bites of the species naturally lacking the c-md ligament being called *BX*. Then, we used discriminant function analysis (DFA) to determine if our kinematic variables can distinguish the different groups. The DFA was performed with the R-package Mass (R Development Core Team, 2011; Riplay et al., 2015). In addition to DFA, we performed a multivariate analysis of variance (MANOVA) (including the seven kinematic variables) followed by pair-wise comparisons (Pillai test) to determine whether *B2* differed significantly from the three other groups (*B1*, *B** and *BX*). We adjusted the significance of the Post-Hoc tests with a bonferroni correction (α/n, with α = 0.05 and n the number of comparisons). The MANOVA and Post-Hoc tests were made in R using the function *skull.manova* (Hothorn and Everitt, 2014; R Development Core Team, 2011).

In the previous study on *A. clarkii*, we showed that there was no difference between *B1*, *i.e.* the pattern performed before c-md transection, and *B**, *i.e.* the pattern performed after c-md transection

Table 2
Principal component analysis on the kinematic data of biting fixed food items in the six species of damselfishes.

	PC1	PC2
Variance explained (%)	47.14	23.50
Eigenvalues	3.30	1.64
1) MRM	0.21	**−0.89**
2) Max. lower jaw depression	−0.46	**−0.64**
3) Mean closing speed of lower jaw	**0.81**	−0.20
4) Peak acceleration in the relative separation of neurocranium and hyoid apparatus	**0.76**	−0.48
5) Time between (1) and the bite	**−0.79**	−0.29
6) Synchronization between hyoid apparatus and lower jaw movements	**−0.70**	0.06
7) Lower jaw closing duration	**−0.82**	−0.29

Results for the two principal component axes: proportions of variance and loading scores of each variable. Important loadings (>0.6) are bolded.

(Olivier et al., 2015). This means that the c-md ligament transection did not affect the kinematics of fish, except to impede $B2$. Consequently, the results of the MANOVA performed on the seven kinematic variables were also used to check whether the $B1$ and B^* patterns were not distinguishable.

3. Results

The PCA revealed two important axes of variation in the kinematic data, explaining 70% of the total variation (Fig. 4). Variables of speeds, acceleration and time load strongly on PC1 (variables 3–7), which explains 47.14% of the variance in the dataset (Table 2). Variables of amplitude (variables 1 and 2) load mainly on PC2 explaining an additional 23.50% of variance (Table 2). The first PC tends to isolate one group of biting events associated with high values along PC1 (Fig. 4). This set of data comprises all the biting events of *S. rectifraenum* before c-md ligament transection and some of the bites of the three other species with c-md ligament (Fig. 4). The associated kinematic pattern was characterised by a rapid movement of both neurocranium and hyoid bar (angular acceleration from 580 to $1904 \times 10^{3\circ}s^{-1}$ in the relative movement between both structures, Table 3) around 12 ± 4 ms before the bite (Fig. 5). During the first 8 ± 2 ms of this movement, the mouth continues to open. Then, the movement of the neurocranium and hyoid bar was accompanied by a fast mouth closing movement; the lower jaw slamming closed within 4 ± 1 ms (Fig. 5, Table 3). The comparison of our data with specimen manipulations and X-ray videos (Parmentier et al., 2007) allowed us to infer that the fast relative separation between the tips of the hyoid apparatus and neurocranium quickly tightens the c-md ligament. Once tightened, the ligament allows a perfect synchronisation between hyoid apparatus and lower jaw movements (variable 6, Table 3). The fast depression of the hyoid apparatus is therefore directly transmitted to the upper part of the lower jaw, closing it in a few milliseconds. We *a priori* classified these biting events as *biting-2* ($B2$). None of the biting events performed by the two species lacking the c-md ligament (*A. troschelii* and *C. chromis*) or by the individuals in which the c-md ligament had been cut were observed in the $B2$ dataset. Contrary to *S. rectifraenum* for which all the biting events observed before the c-md ligament transection were represented in the $B2$ dataset (Fig. 4, Table 1), the three other species with a c-md ligament performed an additional kinematic pattern, which we *a priori* classified as *biting-1* ($B1$). On the PCA scatter plot, this dataset overlapped with the bites performed after the c-md ligament transection (B^*), but also with the bites performed by the species without the c-md ligament (BX) (Fig. 4). During these patterns, movements of the neurocranium and the hyoid apparatus were observed 58 ± 18 ms before the bite (Fig. 5). The peak instantaneous angular accelerations in the movement of
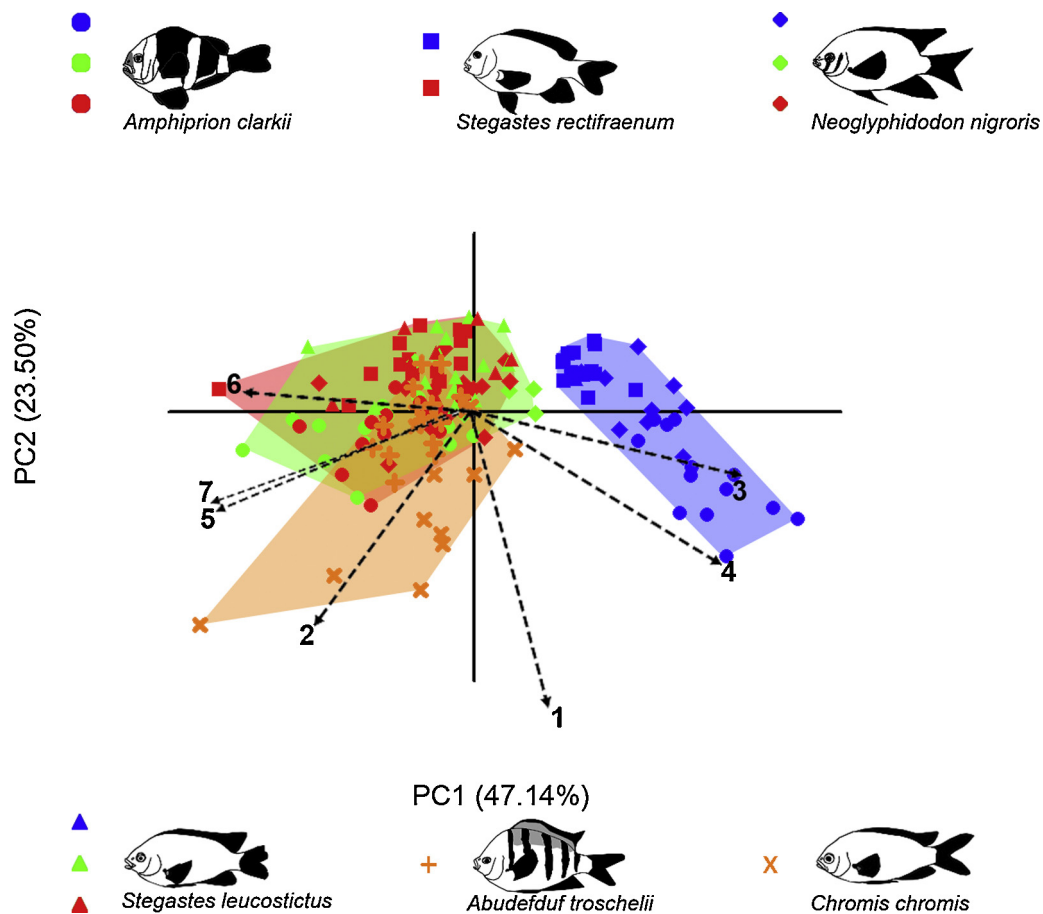

Fig. 4. Plot of principal components 1 and 2 for the biting of fixed food items in six species of damselfishes. The variables that load on each axis are indicated by the arrows, each arrow has a figure representing the variable: (1) maximum relative distance (MRM) between the neurocranium and the hyoid apparatus; (2) maximal lower jaw depression; (3) mean closing speed of lower jaw; (4) peak angular acceleration in the relative separation between the neurocranium and hyoid apparatus; (5) duration separating the time of the bite and the MRM between the neurocranium and hyoid apparatus; (6) synchronisation between hyoid apparatus and mandible movements; (7) mouth closing duration. The green symbols represent *biting-1* ($B1$); the blue symbols represent *biting-2* ($B2$); the red symbols represent the bites after c-md ligament transection (B^*) and the orange symbols represent the bites of species that naturally do not have the c-md ligament (BX). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Mean (±SD) of the kinematic variables measured for the different behaviours in the six species studied. *B1*: biting-1; *B2*: biting-2; *B**: biting after the transection of the cerato-mandibular (c-md) ligament and BX: biting when the c-md ligament is naturally absent.

	A. clarkii			N. nigroris			S. leucostictus			S. rectifraneum		A. troschelii	C. chromis
	B1	*B2*	*B**	*B1*	*B2*	*B**	*B1*	*B2*	*B**	*B2*	*B**	BX	BX
1	11.3 ± 2	17 ± 3.4	10.7 ± 0.9	10.4 ± 1.7	9 ± 1	8.4 ± 1.4	6.7 ± 2.7	9	7.3 ± 2.8	8.2 ± 1.1	7.5 ± 0.6	9.6 ± 2.4	16.1 ± 3.7
2	20.4 ± 4.3	12 ± 1.7	22.4 ± 4.6	16.4 ± 4.6	15.6 ± 6.8	21.1 ± 6.6	17.8 ± 5.4	14.7	16.6 ± 4.7	15.2 ± 1.8	15.2 ± 4.1	25.2 ± 5.1	33.1 ± 8.4
3	434 ± 190	3013 ± 562	422 ± 38	591 ± 270	2969 ± 1474	540 ± 195	394 ± 128	1154	431 ± 115	1688 ± 325	396 ± 65	542 ± 206	694 ± 159
4	187 ± 53	1904 ± 390	189 ± 71	583 ± 152	1073 ± 327	508 ± 131	318 ± 87	866	326 ± 83	580 ± 42	85 ± 18	333 ± 76	628 ± 291
5	26 ± 13	0	28 ± 7	13 ± 9	0.2 ± 0.6	21 ± 12	14 ± 13	0	20 ± 15	0	19 ± 9	23 ± 8	28 ± 13
6	18 ± 8	0.8 ± 1	12 ± 3	10 ± 11	0.4 ± 0.8	13 ± 10	14 ± 10	4	13 ± 12	0.9 ± 1.5	17 ± 9	12 ± 7	10 ± 7
7	46 ± 16	3.4 ± 0.9	43 ± 7	20 ± 14	2.9 ± 1.4	30 ± 10	29 ± 10	6	31 ± 11	4.3 ± 1	39 ± 2	34 ± 8	42 ± 45

1 = MRM between neurocranium and hyoid apparatus) ($°$); **2** = Max. mandible depression ($°$); **3** = Mean closing speed of lower jaw ($°\,s^{-1}$); **4** = Peak angular acceleration in the relative separation between neurocranium and hyoid apparatus ($10^3 °\,s^{-2}$); **5** = Time between (1) and the bite (ms); **6** = Synchronization between hyoid apparatus and mandible movements (ms); **7** = Lower jaw closing duration (ms).

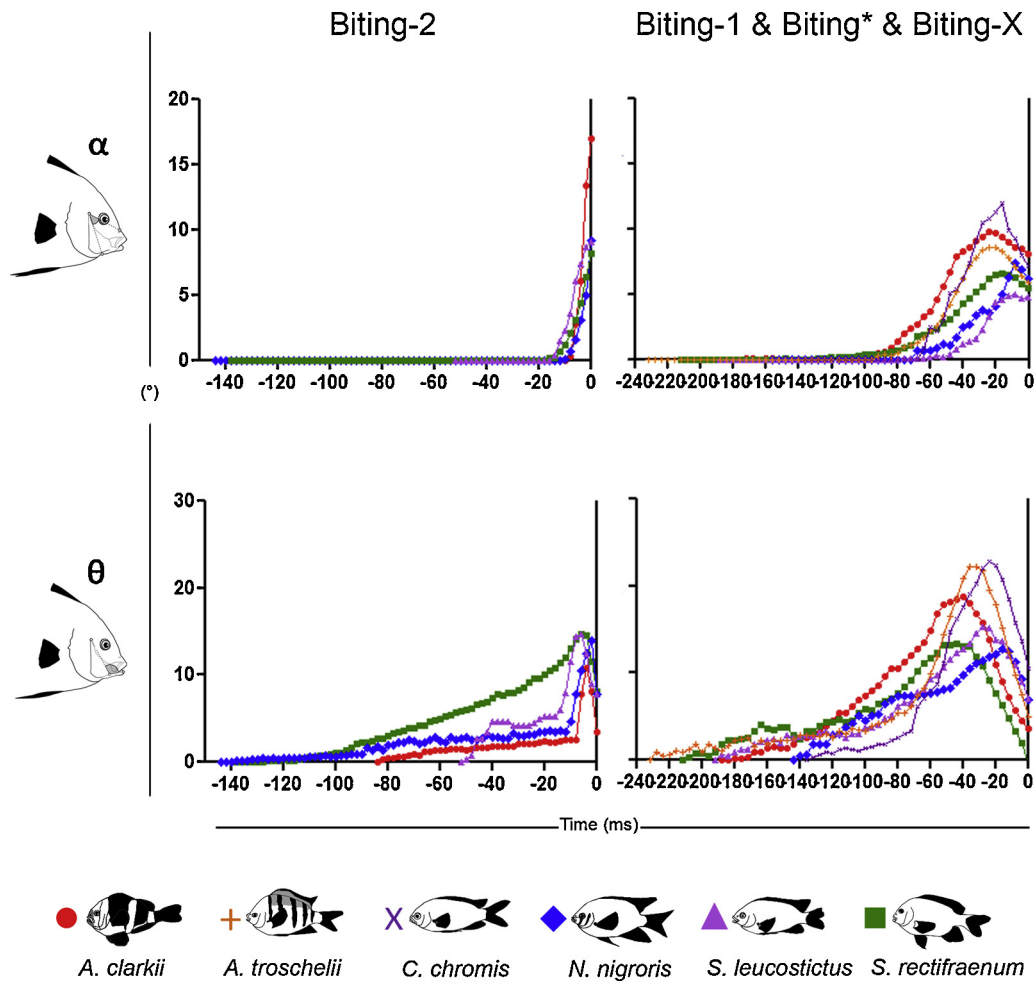**Fig. 5.** Kinematic profiles of the biting patterns in the six species studied. Representation of the angular changes ($°$) over time (ms) describing the relative movement between the neurocranium and the hyoid apparatus (α) and the lower jaw rotation (θ) in the different biting patterns. Patterns start at the onset of the lower jaw depression and end at the time of the bite. Means per species are represented; the standard deviations are withdrawn for clarity.

the neurocranium and hyoid apparatus were never followed by a slam shut of the oral jaws and the mouth was closed much more slowly than in *B2* (36 ± 7 ms vs 4 ± 1 ms; Fig. 5, Table 3).

The DFA, based on the kinematic variables, supported our classification deduced from PCA. All but one *B2* were correctly classified (Table 4). The misclassified biting sequence was one of *S. rectifraenum* for which the lower jaw closing speed was a little bit slow ($\sim 1000 °\,s^{-1}$) in comparison with the *B2* of the other species (Table 3), probably explaining its misclassification. However, all

the *B2* were correctly classified when DFA was run on the *S. rectifraenum* dataset. Contrary to *B2*, many *B1* and *BX* were misclassified as *B** (Table 4).

The MANOVA results revealed a significant difference among the four kinematic patterns ($F_{3,152} = 12.87$, $p < 0.001$). The Post-Hoc tests indicated that *B2* differs from *B1* ($F_{1,71} = 26.74$, $p < 0.001$), *B** ($F_{1,89} = 55.93$, $p < 0.001$) and *BX* ($F_{1,66} = 53.54$, $p < 0.001$). However, Post-Hoc tests did not reveal differences between *B1* and *B** ($F_{1,86} = 1.18$, $p = 0.32$). Thus, the c-md ligament transection did not

Table 4

Discriminante Functional Analysis indicating the ability of the analysis to categorize the bites by kinematic variables. *B1*: biting-1; *B2*: biting-2; *B**: biting after the transection of the cerato-mandibular (c-md) ligament and BX: biting when the c-md ligament is naturally absent.

A priori groups	How the model fit				
	B1	*B2*	*B**	BX	% correctly classified
B1	13	0	19	3	**37**
B2	0	37	1	0	**97**
B*	8	0	42	3	**79**
BX	4	0	8	18	**60**

affect the *B1* pattern, meaning that the surgical operation has no other effect on fish than to impede performing *B2*.

4. Discussion

All the damselfish with the c-md ligament were able to perform a *B2* kinematic pattern but our results do not support that the use of this pattern is related to the trophic habit of the species. *Stegastes rectifraenum*, a species feeding mainly on filamentous algae, performed a *B2* pattern every time (Olivier et al., 2014). In contrast, *Amphiprion clarkii*, feeding mainly on elusive prey, performed either a *B1* or *B2* pattern (Olivier et al., 2015). We expected the two other benthic feeders, *Neoglyphidodon nigroris* and *Stegastes leucostictus*, to perform a *B2* pattern every time as observed in *S. rectifraenum*. That is clearly not the case; both species performed either *B1* or *B2*, similar to *A. clarkii*. This divergence occurring between the two *Stegastes* species may suggest that such a variation is not related to phylogeny although our set of data is too small to draw definitive conclusions. Our results help to refine factors driving the use of one or two biting patterns by damselfishes. The variability is still unexplained but we provide here two hypotheses that may not be mutually exclusive.

In *S. rectifraenum*, the *B2* pattern appeared to be essential to seize small filamentous algae, which could not be seized once the c-md ligament was cut (Olivier et al., 2014). It was argued the c-md ligament is a key trait for the grazing of filamentous algae in Pomacentridae. Grazing damselfishes mainly feed on delicate filamentous algae that are only a few mm long (Hata and Nishihira, 2002). At least two different strategies are possible to seize them: (1) to perform a large bite on the area on which the algae grow; (2) to perform an accurate fast bite to efficiently seize the visually selected algae. Grazing damselfishes are often territorial farmers that manage small algal crops on which they feed (Ceccarelli, 2007; Hata and Nishihira, 2002). Hata and Kato (2002) reported that these farms are often actively maintained for more than 10 years. To keep farms active during these long periods, fish should adopt modes for extracting algae that do not destroy the crops. The large bite strategy is therefore not appropriate for farming because it would inevitably destroy the substrate supporting the crops. This feeding mode is performed by roving herbivorous fish such as Acanthuridae or Scarinae, well-known to remove large amounts of sediment on the reefs (Bellwood and Choat, 1990; Bruggemann et al., 1994; Clements and Bellwood, 1988; Hiatt and Strasburg, 1960; Hixon, 1997; Russ and John, 1988; Vine, 1974) and to destroy damselfish farms in a few days once the resident is excluded from its territory (Hata and Kato, 2003; Lobel, 1980). The strategy of a small and fast bite seems much more appropriate to maintain active farms. Small bites decrease damage to the crop support and fast bites allow an efficient seizure of small and flexible food (slow movement would increase the probability that the algae glides along the lips). The bites of grazing damselfish can be therefore considered as surgical strikes allowing the fish to capture selected small filamentous algae and to manage an active farm (Olivier et al., 2014). Therefore, the

occurrence of filamentous algae in the diet of the fish could explain the frequency at which the *B2* pattern is used. *Amphiprion clarkii* do not feed on filamentous algae and generally captures small zooplanktonic prey by suction (Olivier et al., 2015). *Neoglyphidodon nigroris* and *S. leucostictus* are considered as grazers and farmers (Ceccarelli, 2007; Itzkowitz and Slocum, 1995) but it is possible that subtle differences with *S. rectifraenum* exist in their feeding habits. Stomach contents have to be treated cautiously because they only reveal what the fish has ingested in the few hours before its capture (Frédérich et al., 2016). Nonetheless, data from the literature suggest that *N. nigroris* and *S. leucostictus* would feed on mobile animal prey in higher proportions than *S. rectifraenum*, i.e 3% for *S. rectifraenum*, 36% for *S. leucostictus* and 49% for *N. nigroris* (Kuo and Shao, 1991; Montgomery, 1980; Randall, 1967). The occurrence of a *B2* pattern during biting events of damselfishes could be related to such a gradient from algivory to omnivory. We hypothesize that strict farming species exclusively use the *B2* pattern while pelagic feeders and benthic feeders supplying their diet with animal prey use a mix of *B1* and *B2* patterns during their biting behaviours.

The second hypothesis relies more on what the individuals in our study were habituated to feeding. Among the three benthic feeders, only *S. rectifraenum* was used to feeding on filamentous algae, the two others coming from the marine ornamental trade. *Stegastes leucostictus* and *N. nigoris* were only accustomed to bite on food items fixed to pliers but not filamentous algae. Although both filamentous algae and food items fixed to pliers elicited the same biting pattern in *S. rectifraenum*, the transection of the c-md ligament did not impede the individuals ability to seize fixed food items, by performing a *B** pattern (a *B** pattern was never used to seize filamentous algae) (Olivier et al., 2014). This means that *B2* is not essential to seize fixed food items in pliers, but only for grazing small filamentous algae. This could explain why *S. leucostictus* and *N. nigoris* used either *B1* or *B2*, because *B2* has never been an essential pattern, at least during the last months of their life.

Although it has been clearly shown that the c-md ligament was involved in the mechanism allowing the mouth to close in 2–4 ms (Olivier et al., 2015, 2014; Parmentier et al., 2007), the absence of the *B2* pattern in species lacking the c-md ligament, i.e. *Abudefduf troschellii* and *Chromis chromis*, confirms our previous findings. It is also of interest to note that this inability to perform *B2* is associated with an absence of farming behaviours in all the species lacking the c-md ligament (Frédérich et al., 2014).

5. Conclusion

In the present study, we show that all the damselfish species with a c-md ligament are able to perform a *B2* pattern while the species lacking this trait cannot. However, our data do not support that the use of such a pattern is related to trophic guilds. The benthic feeder *Stegastes rectifraenum* used *B2* every time, while the pelagic feeder *Amphiprion clarkii* and two other benthic feeders, *Neoglyphidodon nigroris* and *Stegastes leucostictus*, did not. As the *B2* pattern is essential to seize filamentous algae in damselfishes, variation in the frequency at which this pattern is used could differ between "specialist benthic feeders" feeding almost exclusively on small filamentous algae and "generalist benthic feeders" that include more mobile benthic prey in their diet. To carry out performance tests to determine whether specialist benthic feeders would be more efficient than more generalist species at grabbing filamentous algae, via the use of only one biting pattern (*B2*), would improve our understanding of the trophic behaviour in Pomacentridae. Ecological variation could greatly influence biting behaviours but we cannot reject a captivity effect. Indeed, except *S. rectifraenum*, all the species came from ornamental trade and were not used to feeding on filamentous algae. This study highlights an intriguing variation

in the use of biting patterns in Pomacentridae. Future investigations should focus on benthic feeders studied directly after their capture from the field and with their natural food (filamentous algae) to shed light on the importance of the *B2* pattern in the feeding behaviour of Pomacentridae.

Acknowledgements

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