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Haliotis tuberculata, a generalist marine herbivore that prefers a mixed diet, but with consistent individual foraging activity

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ABSTRACT
While population foraging behaviour of herbivores has been extensively studied, individual choice is still poorly understood. Very few studies have focused on the individual consistency of foraging behaviour in marine herbivores. Because marine ectotherms are strongly influenced by their environment and because a mixed diet is appropriate for herbivores, we hypothesized that Haliotis tuberculata, a large marine gastropod, would not exhibit significant individual consistency in foraging activity and would display generalist food choices. To test these hypotheses, the behaviour of 120 abalone was studied using a choice test of eight macroalgal species over 3-weeks, with video recording 24 hours a day. In addition, primary components, secondary metabolites and toughness of the eight algae were measured. At the population level, food choice was mainly related to the protein composition and the toughness of the macroalgae. We found that H. tuberculata is a generalist species feeding on a variety of algae (IS = 0.64), even if 21% of the individuals can be considered to be specialists. However, in contrast to our hypothesis, highly consistent between-individual variation was observed in foraging activity (ICC

= 0.81 for time spent feeding and ICC = 0.74 for number of feeding visits per day). The high individual consistency of foraging activity has some ecological and evolutionary implications currently not understood for this marine herbivore.

Keywords: intraclass correlation, proportion similarity index, food choice, marine herbivore, foraging activity, mollusc, individual consistency

INTRODUCTION

Herbivores have to feed on items that are highly variable and often nutritionally much poorer than their own body (Sterner & Hessen, 1994). For herbivores with nutritionally poor prey items, foraging choices are complex, and a mixed diet is a good food choice strategy in terms of nutrition (Day & Cook, 1995). It has been suggested that food choice strategies of herbivores depend on four major components in plants: protein content, chemical defences (distasteful or toxic substances), chemical feeding stimulants (perhaps related to essential nutrients), and physical defences such as the toughness of plant tissue (Scheidel & Bruelheide, 1999). As plants contain much less protein than animals, protein content plays an important role in determining the preferences of herbivores (Mattson, 1980). Protein composition is highly variable between algal species (Jung, Lim, Kim, & Park, 2013; Mai, Mercer, & Donlon, 1994; Mai, Mercer, & Donlon, 1995) and between seasons and harvest sites (Renaud & Luong-Van, 2006; Schmid, Guiheneuf, & Stengel, 2014; Villares, Fernandez-Lema, & Lopez-Mosquera, 2013). Another important component determining food choices in abalone is the chemical defences of macroalgae. In marine gastropods, post-ingestive consequences of secondary metabolites have been evaluated in only a few cases, showing that growth is reduced by secondary metabolites in sea hares (Pennings & Carefoot, 1995) and abalone (Fleming, 1995b; Winter & Estes, 1992). The effect of a third potential determinant of feeding behaviour on marine herbivores, algae toughness, has been quite controversial. Some concluded this is of only minor importance (Shepherd & Steinberg, 1992) while others have observed important effects of such structural defences (Winter & Estes, 1992). However, neither the effectiveness of any single factor nor the interactions between them is yet fully understood. In addition, even if some food preferences can be determined at a population level, individual foraging strategies can nuance these preferences.

Consistency of foraging behaviour can be studied with different approaches, historically separate but conceptually similar. One approach focuses on food resource use to determine if the population and individuals can be defined as generalist or specialist (Mather, Leite, & Batista, 2012). The determination of individual specialisation is important because apparently generalized species might be composed of individual specialists using only a small part of the population's food niche (Bolnick et al., 2003). This variation within the population can be considered as adaptive in itself (Valen, 1965) and can persist over time and across contexts (Toscano, Gownaris, Heerhartz, & Monaco, 2016). Some tools have been developed which allow quantitative assessment of within- and between-individual variation in resource use (Bolnick, Yang, Fordyce, Davis, & Svanback, 2002). Because generalist behaviour might result in ingesting a more optimal set of dietary components for an herbivore with low locomotion abilities, we hypothesized that Haliotis tuberculata would display a generalist algal choice strategy at the population level with non-specialized individual food choices.

A second approach to study foraging behaviour consists in studying individual behavioural consistency and personality. Consistency of individual behavioural patterns across time and between different contexts has been reported in taxa ranging from fish to mammals, with most of the studies done on insects, and focusing on mate preference and courtship (Bell, Hankison, & Laskowski, 2009). Foraging types can be an important factor in determining community dynamics, with a spatial structuration of behavioural type (Griffen, Toscano, & Gatto, 2012). Individual consistency can also be used as a rough estimate of the heritability of a trait (Dohm, 2002; Falconer & Mackay, 1996). As suggested by the lower heritability of morphological traits for ectotherms in comparison to endotherms (Mousseau & Roff, 1987), we expected the heritability and thus indirectly the consistency of foraging activity would be low in abalone. To our knowledge, only a few experiments have been performed on the consistency of foraging behaviour in marine ectotherms, with most of them done on predators (Koteja, Carter, Swallow, & Garland, 2003; McHuron, Hazen, & Costa, 2018; Missoweit, Engels, & Sauer, 2007; Morgan, Hassall, Redfern, Bevan, & Hamer, 2019; Patrick et al., 2014; Toscano & Griffen, 2014), but none of them performed on marine herbivores.

To test these hypotheses, the foraging behaviour of Haliotis tuberculata, a large marine herbivore common in European sublittoral environments, was studied with three objectives: 1) to understand the food choices at a population level in relation to important components of potential prey algae (primary, secondary metabolites and toughness) and diurnal rhythm 2) to evaluate the prevalence of individual specialisation in the population and 3) to evaluate the consistency of foraging activity at the individual level.

MATERIAL AND METHODS

Animal origin and maintenance

Behavioural experiments were carried out at the France Haliotis organic abalone farm facilities (48°36'46N; 4°33'30W) in Plouguerneau, France, between December 2011 and March 2012. Chemical analyses of macroalgae were performed in the Marine Environmental Sciences Laboratory (LEMAR, Brest, France) for phenolic compounds, proteins, lipids and carbohydrates; in the University of Western Brittany (UBO, Brest, France) for nuclear Magnetic Resonance HRMAS analyses (to detect phenolic compounds).

Adult Haliotis tuberculata (65-70 mm total shell length, 4 years old) were reared from systematic mating between wild and farmed broodstocks, mainly to avoid inbreeding. From the age of 1 year until the start of the experiment, they were reared in a cage-structure placed in the subtidal zone. The abalone were randomly harvested from the structure without removing them from the black plastic oyster seed collectors used to provide crevices for them (diameter: 140 mm). They were transported to the land-based laboratory in a 50 L seawater tank. Once in the laboratory, eight abalone were randomly assigned to each experimental tank. A reflective tag was gently glued to each individual with cyanoacrylate glue, without removing the animal from the collectors, to minimize any stress. The tags were designed to allow us to recognize each individual abalone in a tank. Abalone were allowed to acclimate to the experimental tanks for 2 weeks before the start of the experiment.

Materials

The six experimental tanks were grey, flat, square tanks with rounded corners, made of epoxy painted fibreglass (1.10 x 1.10 x 0.20 m, water volume = 100 L), with rough plastic strips on the inside edges to prevent abalone escaping. Each tank held 75 $L.h^{-1}$ of 3 µm mechanically filtered and temperature regulated seawater (12.0 ± 0.5 °C), and was provided with an aeration system. Eight pairs of plastic oyster seed collectors were uniformly distributed along the edges of each tank to be used as hiding places. Eight plastic feeders (7 x 7 x 3 cm) were fixed onto the floor at the center, at equal distances from the eight hiding places, to ensure equal access to each algal species (Figure 1). Five of the tanks contained abalone. The last tank was used as a control to follow natural degradation of the algae. The light:dark regime was 10:14 h (Light: 0830 to 1830 hours; Dark: 1830 to 0830 hours). To avoid stressful conditions during light changes, a transition of light level was programmed over 30 minutes during dawn (0800 to 0830 hours) and dusk (1830 to 1900 hours) using a dimmer (Gold Star, Besser Elektronik, Italia). Tanks were cleaned twice a week using a hose and water filters were changed every day. All tanks were continuously videotaped by five digital cameras (TS-WD6001HPSC, Sygonix Gmbh, Germany) linked to a 24h recording device (TVVR 40021, Abus, Germany).

Experimental procedure

Food choices of abalone were studied using 8 macroalgal species: Asparagopsis armata, Palmaria palmata (Rodophyta), Enteromorpha intestinalis, Ulva lactuca (Chlorophyta), Saccharina latissima, Saccorhyza polyschides, Laminaria digitata and stipes of Laminaria hyperborea (Phaeophyta). Fresh algae were collected at Pors Grac'h beach (48°37'58 N; 4°32'57 W), Plouguerneau, France. The macroalga provided during the acclimation period was Palmaria palmata. From one to four years of age during commercial growth conditions, the eight algae had been provided to these abalone on a regular basis by France Haliotis, with Palmaria palmata and Laminaria digitata being the most important in terms of quantity. After the acclimation period, P. palmata was removed 3 days prior to the start of the experiment.

To determine wet biomass intakes of abalone, the eight macroalgal species were weighed after drying them gently with paper towels and each alga was randomly allocated to one feeder position in each tank. The quantities of algae provided were chosen to provide ad libitum access (at least half of the initial quantity remaining at the end of the period). After 4 days, the remaining algae were removed from each feeder, dried in the same way and wet weighed. Abalone were then starved for 3 days after this food choice trial and the same procedure was repeated during two more weeks to study the food choices and foraging activity over a 3-week period. However, in each tank the algal species were allocated to the same feeder positions during the two subsequent trials. The natural change in weight of algae was measured in the control tank. The quantity of algae ingested by all the abalone in a tank was calculated by removing the change of weight in the control tank from the reduction in weight of the algae in the tanks containing abalone. The 3-week experiment was replicated three times (in total, n = 15 tanks with abalone, n= 3 tanks with only algae).

Food choices and foraging activity analysis

Experimental tanks were filmed 4 days per week from 1830 hours on Monday to 0900 hours on Friday during a 3-week period. The videos were analysed at 16x speed, slowed to 4x at the beginning and the end of each food intake period. The researchers were blind to the alga used in the feeders during video analysis.

Mean feeding bout duration was calculated by subtracting the start feeding time from the stop feeding time for each visit to the feeder, and averaged per day. Total time spent feeding was the sum of all feeding durations per day. The number of feedings was the number of feeder visits done per day. Individual foraging activity was followed using individual reflective tags. These behavioural variables were used for the ICC analysis (see Statistical Analysis for details). For algal preference, variables were calculated per alga for each tank, using the three weeks of individual foraging behaviour. The number of times the abalone chose each of the eight algae during the 3-weeks was recorded, to calculate the proportional similarity index as well as the prevalence of individual specialisation in the population (see Statistical Analysis for explanations).

Algal chemical analysis

Sample collection

Samples from all algal species were collected on the 6th of February and the 5th of March. These samples consisted of the same parts of the algal thalli as were available to the abalone during the food choice experiment. In total, six samples (three per collection day) of 200 g, representative of the algae distributed, were vacuum packed, immediately frozen and stored in a freezer at -20°C before lyophilisation at -55°C for 96h. Dried algae were then crushed, to be used in all the following tests except fatty acid analysis. For fatty acid analysis, samples of 2 g were frozen with liquid nitrogen and stored in a freezer at -80°C before crushing. Dry matter content of each species was determined by weighing the samples before and after freeze-drying.

Algal toughness

Algae were ranked from 1 to 8 according to their toughness (Steneck & Watling, 1982) and visual evaluation of their thickness : 1 being the thinnest and softest alga, and 8 the thickest and toughest alga (1 : Asparagopsis armata and Enteromorpha intestinalis, 3 : Ulva lactuca, 4 : Palmaria palmata, 5 : Saccharina latissima, 6 : Laminaria digitata, 7 : Saccorhyza polyschides, 8 : stipes of Laminaria hyperborea).

Total protein content

Total protein was calculated from total nitrogen content of the samples by Kjeldahl's method, as modified by Connan (2004). The homogenized samples (150 mg) were digested by boiling for 40 min in concentrated sulfuric acid (H_2SO_4), with a catalyst (K_2SO_4+$CuSO_4$+Se). The end product is an ammonium solution. Excess base (NaOH) was added to the product to convert NH_4^+ to NH_3. After distillation in a Büchi (Büchi 323, Büchi Labortechnic, Switzerland), dissolved NH_3 was recovered in 25 ml boric acid (H_3BO_3) solution containing Tashiro's indicator. Titration was performed with sulfuric acid at 0.01M. Analysis of a blank was run in parallel to the analysis of samples. The protein content (%) of the samples was calculated according to the following formula: ((Vs-Vb)*28*F)/W

Where:

Vb = mL titrant for the blank

Vs = mL titrant for the sample

W = Weight of sample in milligrams

F = Factor used to convert nitrogen percentage in a sample to percent protein, its value was 6.25

Total carbohydrate content

Total carbohydrate content (%) was measured by the spectrophotometric orcinol-sulfuric method, using glucose as a standard. Algal powder (5 mg) was diluted in distilled water (5 mL) and vortexed before sampling. Samples (200 µL, 3 replicates) were introduced into glass test tubes, then 400 µL of orcinol-sulfuric reagent (1.5%) and 3 mL H_2SO_4 (60%) were added. The test tubes were placed in a shaking water bath for 20 min at 80°C. The reaction was stopped by placing the test tubes in an ice bath for 2 min and storing them in a dark room for 45 min. The absorbance of all samples was measured at 510 nm using a spectrophotometer (Perkin Elmer).

Total lipid content and fatty acid analysis

Lipid extraction was conducted on 150 to 200 mg of algal powder. Aliquots were transferred in glass test tubes previously heated for 6 h at 450 °C and containing 6 mL of a chloroform/methanol mixture (2/1, v/v), in accordance with the Folch et al. (1957) method. The method is described in detail in Roussel et al. (2019). Quantitative fatty acid spectra obtained by gas chromatography were used to calculate the molar content of each fatty acid in the samples. Depending on the number of double bonds they display, fatty acids (FA) were classified into three groups: saturated FA (SFA, no double bond), monounsaturated (MUFA, only one double bond) and polyunsaturated (PUFA, two or more double bonds). FA could also be differentiated by the position of the first double bond from the terminal carbon: n-3 (omega 3) or n-6 (omega 6). The results used in the PCA were: total lipid content (µg FA / mg dry matter), n-3/n-6 ratio, MUFA content (% of total FA), PUFA content (% of total FA) and SFA content (% of total FA).

Phenolic compounds analysis

NMR HRMAS analysis was performed first to detect the presence of phenolic compounds. Phenolic compound analysis was conducted only on three algal species where phenolic compounds were detected: Asparagopsis armata (Rhodophyta), Enteromorpha intestinalis (Chlorophyta) and Saccharina latissima (Phaeophyta). Extraction of phenolic compounds was conducted in triplicate for each algal species by putting algal powder (400 mg) in an ethanol/water solution (40 mL) for 3 h at 40°C in a shaking water bath. The amount of total phenolics in the 3 species extracts was determined using the Folin-Ciocalteu reagent following the method of Slinkard and Singleton (1977) using gallic acid as a standard for Asparagopsis armata and Enteromorpha intestinalis and phloroglucinol as a standard for Saccharina latissima. Samples (20 µL, 3 replicates for each extract) were introduced into microtiter plate wells, and then 10 µL of Folin-Ciocalteu reagent, 40 µL $NaCO_3$ (7.5%), and 130 µL H_2O were added. The absorbance of all samples was measured at 765 nm using a spectrophotometer (Labsystems multiskan MS) after incubating at 70°C for 10 min. Results were expressed as milligrams gallic acid equivalent (GAE) / g fresh algae sample for Enteromorpha intestinalis and Asparagopsis armata or milligrams phloroglucinol equivalent (PE) / g fresh algae sample for Saccharina latissima per gram of fresh weight.

Statistical Analysis

To study the general foraging activity of the population, the total number of feeder visits was calculated over all the abalone in each tank during the day and night periods over the 3 weeks (N = 15 tanks in total). To compare the number of day versus night feeding visits, a Wilcoxon signed rank test for paired data was used because the conditions to use a parametric test were not fulfilled. In addition, the total number of feeding bouts initiated in each 2-hour period of the 24-hour cycle was calculated per tank.

To study the population food choices, a Freidman two-way analysis of variance by ranks was used. Behavioural variables were calculated per alga for each tank over the three weeks. This non-parametric analysis was proposed by Lockwood (1998) and Siegel and Castellan (1988) for multiple-choice feeding preference experiments. A rank of 1 corresponded to the algae eaten in largest quantity while a rank of 8 corresponded to the algae eaten in the smallest quantity (Table 1). Post-hoc comparisons were performed with the method proposed by Siegel and Castellan (1988).

Spearman correlations with adjusted p-value (Holm's correction) were performed to study the relationships between the foraging behaviour per algae for each tank over the three weeks and the chemical variables.

For each individual, the consistencies of their mean feeding bout duration, their total time spent feeding per day and their number of feeding bouts per day, were studied over the three weeks. The four daily values (k = 4 days per week) were averaged per week and per individual. Prior to calculating repeatability statistics, the data were log-transformed when non-normality of residuals was detected (Shapiro's test) or when variances were found to be significantly different (Levene's test) to satisfy the assumption that there is a common population (residual) variance (Biro & Stamps, 2015). Thereafter, ICC estimates for the transformed foraging variables and their 95% confidence intervals were calculated using the psych package in R (Revelle, 2018), based on a mean-rating (k = 4), consistency-agreement, 2-way mixed-effects model (weeks are considered fixed effects but individuals are treated as random effects) with 120 individuals.

A proportional similarity index (PS) was calculated for each individual as well as the prevalence of individual specialisation in the population (IS) using the RInSp R package (Zaccarelli, Mancinelli, & Bolnick, 2013) to study whether abalone consistently act as specialists or generalists with respect to algal choice. Abalone which did not feed at least four times during the 3-week experiment (18 abalone out of 120) were not used for this individual specialisation analysis.

Ethical Note

Manipulation of individuals involved food limitation and diet modification. At the end of the experiment, the abalone were removed from the tanks and placed in another farm husbandry structure.

RESULTS

Nocturnal foraging activity

The abalone foraging behaviour varied between the day and night: the total number of feeder visits was much higher during the night than during the day periods (night vs day period: 91 ± 9.4 vs 13 ± 2.4 visits per tank over the 3-week period, Wilcoxon signed rank test for paired data, $N = 15$, $P < 0.001$) (Figure 2).

Food choices of the population

The total quantity ingested varied between algal species (Freidman two-way analysis of variance by ranks, $Q = 48.4$, $N = 15$, $P < 0.001$) (Figure 3): Enteromorpha intestinalis was the most ingested alga, followed by Asparagopsis armata. On the other hand, Laminaria digitata and stipes of Laminaria hyperborea were the algae ingested in the smallest quantities. Ulva lactuca, Saccharina latissima, Saccorhiza polyschides and Palmaria palmata were ingested in intermediate quantities, not significantly different from the most ingested alga or the least ingested alga.

The Freidman two-way analysis of variance by ranks showed that there were significant differences between algal species in the number of feeding visits per day ($Q = 45.7$, $N = 15$, $P < 0.001$), the total time spent feeding each day ($Q = 42.4$, $N = 15$, $P < 0.001$) and the mean feeding bout duration ($Q = 36.7$, $N = 15$, $P < 0.001$) (Figure 3). However, the ranks for the algae were different depending on the variable (Figure 3): for example, Enteromorpha intestinalis was the alga ranked first in terms of quantity ingested and for the number of visits, but was ranked fifth for the mean feeding duration; while Saccharina latissima was ranked respectively fourth and fifth for the quantity ingested and number of feeding visits, but second for the mean feeding bout duration.

Food choice in relation to algal characteristics

The quantity of algae ingested per day as well as the number of feeding visits per day were significantly correlated to the protein content of the algae (respectively, $r_s = 0.40$ and $r_s = 0.54$) and negatively correlated to algal toughness (respectively, $r_s = -0.50$ and $r_s = -0.51$) (Table 1). In addition, the number of visits per day was positively correlated to total lipid content ($r_s = 0.35$), n-3/n-6 ratio ($r_s = 0.35$) and negatively correlated to MUFA content ($r_s = -0.34$). No correlation was observed between the mean feeding bout duration and any of the algal chemical components. The total time spent feeding was correlated to the total protein content ($r_s = 0.39$) and to the total lipid content ($r_s = 0.33$).

Individual and population specialisation

The H. tuberculata population consumed resources in a similar way, with a specialisation index IS of 0.64 ($p < 0.001$). However, 21% of the individuals had a PS < 0.50 (Figure 4)

Individual foraging activity consistency

Very high consistency of individual foraging activity was observed for the total time spent feeding per day (ICC = 0.81, $F_{119,238} = 5.2$, $p < 0.001$, 95% confidence interval = 0.74 – 0.86), as well as for the number of feeder visits per day (ICC = 0.74, $F_{119,238} = 3.8$, $p < 0.001$, 95% confidence interval = 0.64 – 0.81, log transformed data) and for the mean feeding bout duration (ICC = 0.74, $F_{119,238} = 3.8$, $p < 0.001$ $p < 0.001$, 95% confidence interval = 0.65 – 0.81, log transformed data).

DISCUSSION

The aim of the experiment was to understand the foraging strategy of H. tuberculata, a marine herbivore at both a population and an individual level. The food choices of H. tuberculata are mainly correlated to the protein composition and the toughness of the macroalgae at the population level. The population was composed mostly of generalist individuals with a few specialist individuals. Surprisingly, foraging activity of individuals is highly consistent.

Nocturnal foraging activity

Foraging activity was mainly observed at night. Foraging activity was intense soon after sunset and progressively increased until 22:00 to reach a stable level between 22:00 and 02:00. Nocturnal feeding and movement has also been reported for other abalone species in laboratory studies (Momma & Sato, 1970; Tahil & Juinio-Menez, 1999) and field conditions (Day & Branch, 2000; Shepherd, 1973; Wood & Buxton, 1996). Even if light intensity is probably the main cue to coordinate abalone circadian behaviour (Morikawa & Norman, 2003), a circatidal clock due to tides cannot be excluded (Wilcockson & Zhang, 2008). There was no obvious sign of tidal variation however, during this laboratory experiment.

Food choices of the population

Although local algal abundances may be the overriding factor determining the diet of abalone in a particular area in the wild (Tutschulte & Connell, 1988; Wood & Buxton, 1996; Zeeman, Branch, Peschak, & Pillay, 2012), as in many laboratory studies, the abalone we studied preferred some algae over others. Enteromorpha intestinalis, with Ulva lactuca and Asparagopsis armata were the preferred algae in term of quantity of algae ingested and numbers of feeding visits. *Laminaria hyperborea* stipes were the least attractive. However, the total time spent feeding and the mean feeding duration give additional information on abalone feeding behaviour. For example, *Asparagopsis armata*, the second most ingested algae in terms of quantity, is at the sixth position for the total time spent feeding and at the seventh position for the mean feeding duration. It is clearly easily consumed.

Food choices based on primary compounds and toughness of algae

Optimal foraging theory implies that organisms should consume foods that maximize fitness by selecting the most energy-rich and nutritional balanced items (Pyke, Pulliam, & Charnov, 1977). The three most ingested algae, *Enteromorpha intestinalis*, *Ulva lactuca* and *Asparagopsis armata* had the highest protein contents, while the lowest protein value was associated with the least ingested alga, stipes of *Laminaria hyperborea*. This experiment shows that the total quantity of an alga ingested and total number of feeding visits were correlated with total protein content but also other parameters such as algal toughness. This is consistent with an emphasis on protein content in abalone food choice and the lower importance of total carbohydrate (Fleming, 1995a). Energy in the form of available carbon compounds is generally thought to be in ample supply in plants and seaweeds because of photosynthesis, but this is not the case for nitrogen compounds, considered to be a more limiting factor. Abalone species have a low lipid requirement (< 5% algal dry weight), typical of herbivorous molluscs and fish (Viera et al., 2005). Total lipid content was only weakly related to food choices. No correlation was found between the level of phenolic compounds and *Haliotis tuberculata* food choices in our experiment, in contrast to the expected avoidance of algae with phenolics (Shepherd and Steinberg 1992). This might be related to the very low phenolic contents observed during this winter period, in the algal species analysed. Effects on feeding behaviour might have been more important during spring or summer, when algae phenolic contents are higher and probably would serve as feeding deterrents or inhibitors for abalone (Fleming, 1995b; Stepto & Cook, 1996).

Algae toughness has been identified as a factor limiting seaweed consumption (Toth & Pavia, 2007). Some experiments have avoided this factor by mixing powdered algae with agar or carrageenan, to remove its effect (Angell, Pirozzi, de Nys, & Paul, 2012; McShane, Gorfine, & Knuckey, 1994). Abalone feed using a rasp-like radula, and the effectiveness of the radula teeth is strongly influenced by the form and toughness of the food items. In our experiment, it seems that the morphological structure of the Haliotis tuberculata radula allows them to graze more effectively on filamentous and thinner algae such as Asparagopsis armata and Enteromorpha intestinalis than on the less favoured thick and tough Laminaria leaves and stipes.

Consistent foraging activity

Highly significant foraging activity repeatability was observed during this experiment. Obtaining accurate, reliable and repeatable phenotypic information is critical for better understanding of how the personalities of individuals can be maintained under environments with different selective pressures (Sih, Bell, & Johnson, 2004). At a population level, in contrast to our hypothesis, foraging activity was very repeatable. In a meta-analysis performed on a wide range of species, the average repeatability of behaviour was reported to be 0.37 (Bell et al., 2009). Foraging behaviour repeatability was reported to be higher (Bell et al., 2009; McHuron et al., 2018) but nevertheless ranged between 0.2 and 0.5 most of the time (Koteja et al., 2003; McHuron et al., 2018; Missoweit et al., 2007; Morgan et al., 2019). In our experiment, the high ICC value and narrow 95% confidence interval for abalone were probably obtained because the mean values for four days per week of a large number of individuals were analysed, sufficient to study repeatability with precision (Wolak, Fairbairn, & Paulsen, 2012). This result highlights that high consistency of individual foraging activity can be observed for abalone, indicating that this herbivore has a rigid foraging activity, at least in winter. The number of feeding visits per day might be a foraging variable related to intrinsic factors (such as active or passive "type" personality). The total time spent feeding per day and mean feeding bout duration have a lower ICC and might be more influenced by extrinsic factors, such as the toughness and other properties of the algal species eaten.

Individual and population specialisation

H. tuberculata is a generalist that feeds on a variety of algae (IS = 0.64). Most individuals were opportunistic, feeding on a large range of algae. But 21% of the individuals can be considered as specialists. Based on our observation of the data, some specialist individuals might choose the same algae because they have a greater appetence for some physical attribute (e.g. the most tender algae such as A. armata and E. intestinalis) or chemical attribute (e.g. the richest in protein such as P. palmata). However for others, no trend can be easily observed. Because some abalone have homing behaviour, their choices might be related to the algae that are closest to their hiding place. Each abalone presumably evaluated the food differently and made decisions to select a suitable diet. Individual differences can result from intrinsic factors (metabolism or individual experience) or extrinsic factors (intraspecific competition or predation). Because these herbivores were reared in the same sea-cage for three years, their previous individual experiences in relation to their rearing environment are probably similar. Metabolism might be an important factor influencing the differences in feeding preference (Hawlena, Hughes, & Schmitz, 2011; Holtmann, Lagisz, & Nakagawa, 2017). Components of the algae previously consumed are also likely to influence future preferences, to gain nutrients lacking in the past diet (Day & Fleming, 1992). In addition, it should be noted that this experiment was performed in a laboratory context with a simplified environment. In the wild, individuals have far more complex decisions to take, depending of the external situation such as the presence of predators or hiding places, algal composition and quantity, etc…

Choosing a mixed diet is probably the best compromise for large marine herbivores, consistent with nutritional advantages and conferring significantly higher fitness than the average of single-species diets, but not for the best single prey species (review by Lefcheck, Whalen, Davenport, Stone, & Duffy, 2013). In a one-year experiment, we have confirmed the advantage of a mixed diet foraging strategy on Haliotis tuberculata, showing that the mixed diet was the second best strategy in terms of growth and reproductive development after a monospecific diet of the best single alga, P. palmata (Roussel et al., 2019). In the wild, P. palmata is often likely to be unavailable. By mixing different sources of algae, a multi-algal diet would allow the acquisition of vitamins, essential fatty acids and amino acids present in different proportions in various algal species. This result fits well the optimal diet theory in the sense that the mixed diet will produce the highest fitness, even if the algae chosen do not have the highest energy value individually (Sih & Christensen, 2001).

CONCLUSION

H. tuberculata is a generalist species in term of algal choice, with a small proportion of the individuals being specialists and the majority being generalists. In addition, individual foraging activity pattern was highly consistent over time, with some abalone feeding every night while others fed only rarely. This study provides a new perspective on the foraging strategy of an abalone species – a very widespread group of large herbivorous marine molluscs. Algal preferences probably result from a compromise between the most useful algae from a nutritional point of view, the physical ability of abalone to consume the food item and some individual consistency. In a further study, it would be interesting to test the interaction of abalone personality traits such as activity and exploration in a novel environment in addition to abalone food resource use, to understand how variations among individuals contribute to patterns at the population level (Toscano et al., 2016).

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Table 1: Correlation between the primary components, secondary metabolites and toughness of 8 algal and the foraging behaviour of 15 groups of 8 abalone. The correlation was performed on the average foraging value obtained per algae for each group of abalone four days per week during a 3-week period. Significant correlations are highlighted in bold

	Quantity of algae ingested per day (g / day)	Time spent feeding per day (min / day)	Number of feeding visits per day	Mean feeding bout duration
Dry matter content %	-0.34 (p = 0.01)	-0. 04	-0.15	0.05
Algal toughness (rank)	-0.50 (p < 0.001)	-0.28	-0.51 (p < 0.001)	-0.00
Total protein content (%)	0.40 (p < 0.001)	0.39 (p < 0.001)	0.54 (p < 0.001)	0.12
Total carbohydrate content (%)	-0.14	0.01	0.12	-0.10
Total lipid content (µg FA / mg dry matter)	0.10	0.33 (p = 0.01)	0.35 (p < 0.01)	0.18
n-3/n-6 ratio	0.13	0.24	0.35 (p < 0.01)	0.06
MUFA content (% of total FA)	-0.21	-0.12	-0.34 (p = 0.01)	0.10
PUFA content (% of total FA)	0.01	0.21	0.17	0.14
SFA content (% of total FA)	-0.05	-0.20	-0.09	-0.19
Total phenolics (mg GAE or PE / g fresh algae sample)	0.41 (p < 0.001)	0.14	0.26	0.02

Figure 1: Design of the experimental tank

Figure 2. Number of feeder visits initiated each 2-h period of the 24-hour cycle of Haliotis tuberculata. Eight abalone per tank were observed for 4 days per week during a 3-week period in a total of 15 tanks. Mean ± s.e.

Figure 3. Algal preferences of abalone. Foraging behaviour was followed four days per week during a 3-week period in 15 tanks containing 8 abalone

Figure 4. Proportional similarity index (Psi) frequency in a population of 102 abalone studied during 3 weeks. Abalone had the possibility to choose between eight different algae in a tank.