ORIGINAL ARTICLE

The influence of season and the tidal cycle on division of labour by the radula during feeding in the estuarine brooding gastropod *Crepipatella dilatata* (Calyptraeidae)

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Abstract

The brooding gastropod *Crepipatella dilatata* can feed by scraping the substrate with the radula and by suspension-feeding, which also requires use of the radula. There is a "division of labour" for the radula among three discrete tasks associated with feeding: (1) removing mucous balls from the food pouch; (2) transferring the mucous cord from the neck channel to the mouth (both components of suspension-feeding); (3) scraping the substrate. We hypothesised that the proportion of time used for each feeding activity varies according to environmental conditions. Total radular activity in females was greatest at high tide and in summer. The rate of radular extrusion for ingesting the mucous cord varied seasonally and between brooding and non-brooding females. Non-brooding females exhibited higher rates of radular extrusion for ingesting the mucous cord and for scraping the substrate than did brooders. In females, radular activity in removing the mucous ball from the food pouch was strongly influenced by the tidal cycle during winter, reaching minimum values at low tide. Differences were recorded in substrate scraping among seasons and within tidal cycles, and among males, brooding females and non-brooding females. Brooding females displayed less rasping than non-brooders, since the area available for grazing was restricted by the egg mass. Throughout the year, including low salinity periods, males allocated a greater proportion of total radular activity to rasping than to removing the mucous ball or ingesting the mucous cord. The feeding behaviour of both males and females is modulated by salinity, but the principal determinants of radular activity are the mode of reproduction (brooding in females) and, in males, motility.

Keywords: *Crepipatella*, Brooding, Estuary, Feeding cycles, Tidal cycles, Radula

Background

Many marine organisms are constantly exposed to fluctuations in environmental factors such as temperature, salinity, hydrostatic pressure, light intensity and food availability over various time scales [1–3]. This is most evident in shallow coastal zones, especially estuaries, where environmental variation can change rapidly owing to tidal cycles and stochastic atmospheric forces (wind, precipitation) that have a major impact on the water column as a result of the relatively small volumes of water in the system [4]. Temporal variation in factors such as water temperature, salinity, suspended particulate matter and oxygen concentration in bays and estuaries is well known [1, 5–9] and generally results in physiological and behavioral responses by organisms living in these environments [10–13]. Salinity is one of the most important environmental variables in estuarine systems [14–16] and may be a significant source of stress for estuarine invertebrates and other organisms [11, 13], particularly sessile forms that are unable to move to more favorable locations [17].

Physiological functions in suspension-feeding estuarine invertebrates are influenced by factors such as

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temperature, salinity, quality and quantity of the seston and exposure to air, most of which are entirely or partially determined by the tidal cycle [1, 7, 8, 11, 13, 18]. The concentration and quality of suspended particulate matter and their role in regulating feeding and ingestion rates has been studied extensively [19–21].

In suspension-feeding marine gastropods such as calyptraeids, the radula is used as a supplementary feeding structure which collects food by rasping the substrate [22–24], a process which is relatively more important in motile than sessile individuals (*Crepidula fecunda*; [25]). The radula, however, also plays a role in suspension-feeding because it is responsible for transferring the mucous cords containing sequestered particles from the gill margin to the mouth via a food canal in the neck [23, 24, 26, 27]. A third feeding function of the calyptraeid radula is the removal of the mucous balls formed in the food pouch, a depression located in the frontal region of the mantle [28–30], either to the mouth for ingestion or to the mantle rim for rejection as pseudofaeces [23, 27, 30]. The material in these mucous balls is acquired by suspension-feeding and originates either on the branchial filaments closest to the head of the animal or from the lateral canal of the mantle, which moves particulate material from the inhalent area of the mantle cavity [23, 24, 27].

Calyptraeid gastropods brood their young within capsules [23, 31, 32] which are affixed to the substrate by the sessile female, ventral to the neck, and are irrigated by the inhalent water current [33]. The protandric hermaphroditic calyptraeid *Crepipatella dilatata* is common in the subtidal and intertidal zones of some estuaries in southern Chile; individuals < 18 mm shell length are male and also motile, whereas those > 18 mm long are female and sessile [34]. The female incubates the capsules until the juveniles are released (direct development; [32, 35]). This species lives in an environment in which variation in salinity and seston is high, owing to high winds and rainfall in winter and spring [7]. In order to minimize osmotic stress to the soft tissues and to the capsules, the female seals the shell to the substrate when the salinity falls below 22.5, preventing entry of water to the mantle cavity and thereby curtailing suspension-feeding [8, 17]. There is little information on the action of the radula, particularly in relation to environmental variables, although it is more associated with motile individuals (all males and juveniles) than with sessile individuals (females), in which the scraping action of the radula is reduced and suspension-feeding is the principal means of food acquisition [23, 25]. Males also isolate the mantle cavity from the exterior environment to prevent osmotic stress to the soft tissues at low salinity, although the critical salinity value [19] is lower than for females. Although isolation of the mantle cavity prevents suspension-feeding, it is not clear whether the radula continues to scrape the substrate, either in males or females.

The three modes of action of the radula in feeding by calyptraeid species (manipulation of the mucous cord, removal of mucous balls from the food pouch and scraping the substrate) vary in importance according to the stage of development [23, 24, 27] and to environmental conditions [36]. In *C. dilatata* suspension-feeding continues uninterrupted at salinities above 22.5 [8, 17], and the radula plays a role by transferring the mucous cord to the mouth. Factors other than salinity may also be important, however, such as the characteristics of the water column, mobility or sessility of the individual, sex and, for females, reproductive condition (incubating or non-incubating). The objective of this study was to determine the roles played by the radula in feeding in females (brooding and non-brooding) and males during various stages of the tidal cycle in different seasons of the year. In particular, the study contributes to an understanding of the impact of temporal changes in the water column on the radular feeding behaviour of an organism that is sessile as female and therefore unable to escape adverse conditions. Furthermore, the paper explores the dependence of feeding responses on the developmental stage of the individual and, for females, the brooding status (brooding or non-brooding).

Methods

Collection and maintenance of specimens

Female and male individuals of the slipper limpet *Crepipatella dilatata* (shell length 25 mm ± SD 2 and 12 mm ± SD 3, respectively) were collected from the Quempillén estuary, Chiloé Island, Chile (41°52′S, 73°46′W), in which salinity can fluctuate between a maximum of 32 at high water in summer to a minimum of 6 at low water in winter [7, 37]. The estuary has a maximum depth of 2 m, and most of the limpets inhabit the shallow sublittoral. All specimens were removed from the natural substrate, allowed to settle on transparent glass plates and maintained in the laboratory for several days in tanks of recirculating seawater pumped from the estuary. Once firmly affixed to the glass plates the specimens were transferred to the estuary at the location from which they were collected, remaining there for at least 2 months, before they were used in experiments. During this period, many of the females began to brood capsules, and brooders and non-brooders were identified.

Radular activity was examined in experimental animals during three 12 h tidal cycles (from high tide to high tide) within three to four consecutive days (to minimise temporal variation in factors such as photoperiod) in each season (spring, summer, autumn and winter). The tidal cycle in the Quempillén estuary is semidiurnal.

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Video recordings were made of radular activity in female limpets (brooding and non-brooding) and males, and salinity, total particulate matter (TPM) and particulate organic matter (POM) in the estuary were measured at the same time as radular activity. Since the laboratory was located on the shore of the estuary, the experimental animals in the aquaria experienced the same conditions as those in the estuary. The hose used to pump water was kept in air until required for an experimental run, therby avoiding the presence of suspension-feeding organisms in the hose and the possibility of a reduction in the particle levels reaching the experimental tanks. During the 24 h period preceding a set of measurements, several experimental animals, previously allowed to settle on transparent glass plates, were removed from the estuary and held in the laboratory in continuously flowing water taken directly from the collection site. They were therefore exposed to the natural environmental conditions, including suspended particulates (no supplementary material was added). Photoperiod and water temperature were not controlled in the laboratory and remained at natural ambient levels.

Salinity

Salinity was determined by placing the sensor of a conductivity meter (YSI) in the tank (10 L) that distributed the flowing water obtained directly from the estuary (ca. 20 m away) to the experimental aquaria. Water was taken close to the bottom, directly above the *C. dilatata* bed. Measurements were made hourly, during the daytime, for three complete random tidal cycles (12 h) over a period of three or four consecutive days in each month selected: January (summer), May (autumn), August (winter) and November (spring). The first measurement was made at high water during the morning in all cycles.

Seston

Water samples for seston analysis were taken in triplicate on the same schedule as the salinity measurements. On each occasion a volume of 200–1000 mL was taken from the distribution tank, depending on the turbidity of the water coming from the estuary. Glass-fibre filters (Whatman GFC, 47 mm diameter) were prepared by pre-washing with distilled water to remove loose fibres, combusted in a muffle furnace (3 h at 475 ℃), transferred to a desiccator and weighed ($\pm$ 0.01 mg) when cool. Each water sample was passed through a prepared filter under gentle vacuum, salts being removed by a brief rinse with distilled water. Loaded filters were dried at 60 ℃ (24–48 h), transferred to a desiccator and weighed when cool. Total particulate matter (TPM) was obtained by difference between the filter + dry seston weight and the filter weight. Dry filters were then combusted (3 h at 475 ℃),

cooled in a desiccator and immediately reweighed. Particulate inorganic matter (ash) was obtained by difference between the filter + ash weight and the filter weight. Particulate organic matter (POM) was calculated by subtracting ash from TPM.

Radular activity

Several specimens (males as well as incubating and non-incubating females) previously allowed to adhere to glass plates were removed from the estuary and placed individually in small glass aquaria (25 × 20 × 25 cm) containing circulating water continually pumped from a point in the estuary only 10 m from the bed from which the experimental animals were taken. The flow of water through each tank was sufficient to ensure that levels of particulate matter, and hence food availability for suspension-feeding, were not significantly depleted by filtration activity of the experimental animals. A dissecting microscope with a video camera and recorder was set up to allow the basal-anterior region of the specimen to be observed continuously throughout each tidal cycle (12 h) through the glass plate to which the specimen was attached. Continuous video recordings were made on 10 brooding females, 10 non-brooding females and 14 males throughout each tidal cycle during each season. These video sequences were used to quantify radular activity in each individual for a complete tidal cycle (12 h). The frequency with which the radula was extruded from the mouth (extrusions per hour) was recorded for each of the three activity categories (transfer of the mucous cord to the mouth, removal of mucous balls from the food pouch and scraping the substrate) in each individual by analysis of the video sequences. Observations for females (brooding and non-brooding) and males were made on the same schedule as the salinity and seston measurements, except in summer, when there were no recordings for male specimens. Each specimen was used during only one tidal cycle.

Statistical analysis

Since radular activity was measured on the same individually tagged animals throughout the tidal cycle, the data were analysed by analysis of variance with repeated measures after verifying that values were normally distributed and the variances homogenous. For the environmental data, three separate two-way repeated measures analyses of variance were carried out with tidal cycle and season as fixed factors in each case and salinity, TPM and POM as dependent variables in the respective analyses. For data on radular activity in females, a three-way design was used with tidal cycle, season and brooding status (brooding vs. non-brooding) as fixed factors and frequency of radular extrusion as the dependent variable.

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Separate analyses using this model were carried out for each type of radular action (mucous cord, mucous ball and substrate rasping). Data for radular activity in males were analysed in the same way, except that the design was two-way, since the fixed factor incubation status was not required. The significance level was set at $p = 0.05$.

Results
Environmental variables
Highly significant differences were observed in water salinity among seasons and within individual tidal cycles ($p < 0.0001$, Table 1A), but there were no significant differences in TPM (Table 1B) or POM (Table 1C). During summer, salinity was high [28–32] throughout the tidal cycle, the lowest values occurring at low tide (Fig. 1a). There was little change in TPM or POM during the tidal cycle, although values for both were lowest just before low water. In the autumn, salinity followed the same pattern as in summer (Fig. 1b), but TPM and POM were lower than in summer and were consistent throughout the tidal cycle. Pronounced changes in salinity were observed during the tidal cycle in winter (Fig. 1c), values falling from 30 at high water to 14 at low tide before rising during the flood. Values for both TPM and POM were higher in winter than in autumn but similar to summer values, and there was no clear tidal effect. The strong influence of the tidal cycle on salinity observed in winter continued through the spring (Fig. 1d), although values did not decrease below 20. Once again there was no

change in TPM or POM during the tidal cycle, and values were similar to those obtained in autumn (Fig. 1b).

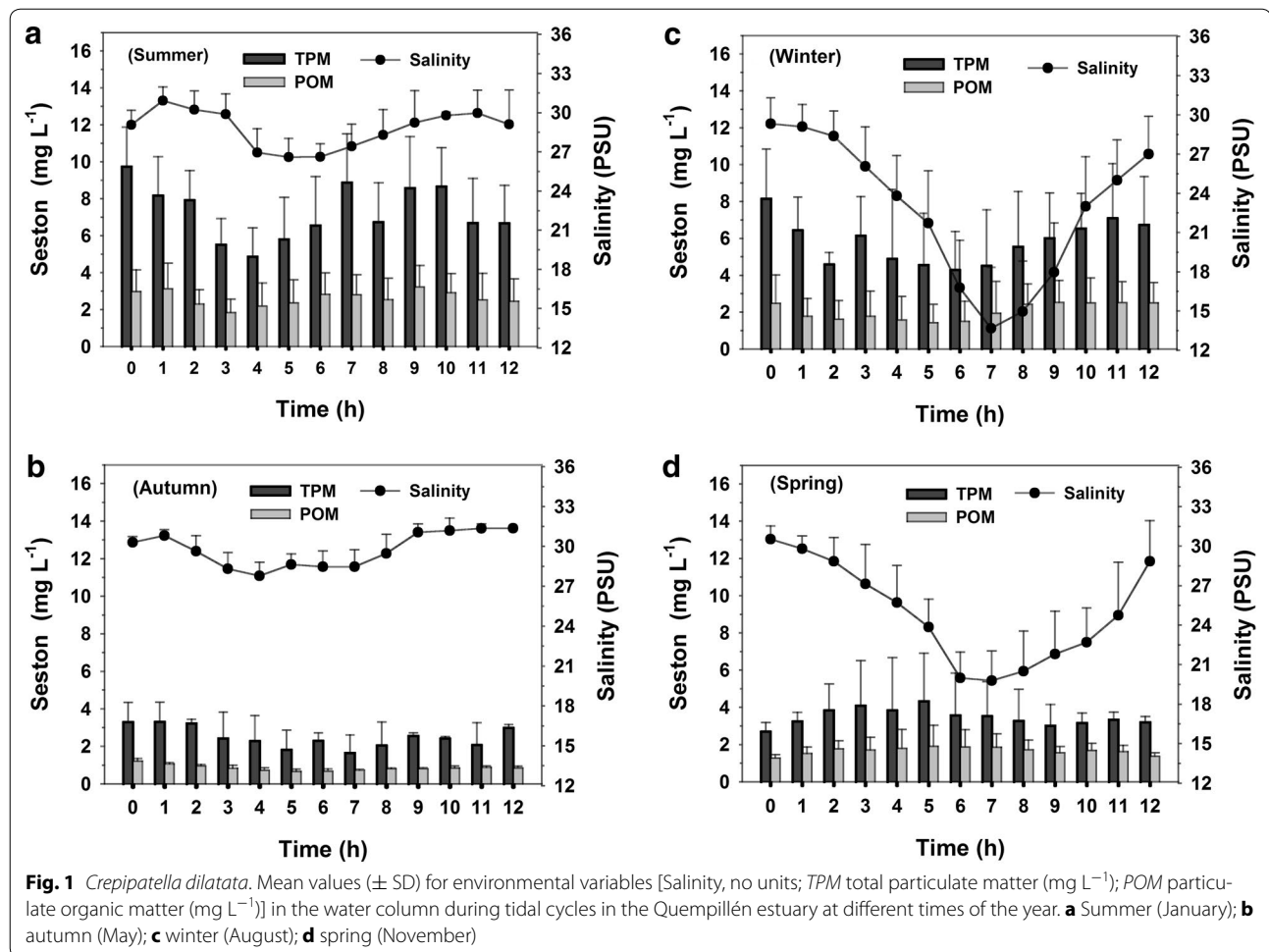### Radular activity in females
Mucous ball
Radular activity in removing the mucous ball from the food pouch differed significantly among seasons ($p = 0.0253$, Table 2A) and within individual tidal cycles ($p < 0.0002$), and the interaction term was also significant ($p < 0.0001$), but there was no significant effect of reproductive condition. During summer, autumn and spring there was no influence of the tidal cycle on the activity of the radula in extracting the mucous ball from the food pouch of females (Fig. 2a, d, j), and no difference between brooders and non-brooders. Mean values overall ranged from 5 to 15 radula extrusions per hour. In winter, however, radular activity was strongly influenced by the tidal cycle in both brooding and non-brooding females (Fig. 2g), falling rapidly during the early phase of the cycle in non-brooders to reach minimum values at low tide in both groups before increasing as the tide rose. Apart from the period during and immediately after low tide (2 h for brooders, 4 h for non-brooders), radular activity associated with the mucous ball was much higher than at other times of the year.

Mucous cord
The rate of radular extrusion for transporting the mucous cord to the mouth differed significantly among seasons ($p < 0.0001$, Table 2B) and within individual tidal cycles ($p < 0.0001$, Table 2B), and the interaction term was also significant ($p < 0.0001$, Table 2B), but there was no significant effect of reproductive condition. There was no effect of the tidal cycle on radular activity during summer in brooders or non-brooders (Fig. 2b), but values were consistently higher in non-brooders. In autumn there was also no clear tidal influence or difference between brooders and non-brooders, but values for non-brooders were much lower than in summer. Radular activity was influenced by the tidal cycle in winter, however, especially in non-brooders, and minimum values occurred at low tide. Values for brooders were lower in winter than in the other seasons of the year. The pronounced tidal effect was also observed in spring, although there was no difference between brooding and non-brooding females, variance in the data being much greater than in winter (Fig. 2h, k).

Rasping
Radular activity in rasping the substrate differed significantly among seasons ($p = 0.0453$, Table 2C), between brooders and non-brooders (i.e. reproductive status; $p < 0.0001$) and within individual tidal cycles ($p = 0.011$).

Table 1 Effects of the tidal cycle (12 h, 13 samples including 0 h) and season (summer, autumn, winter and spring) on the environmental variability of the water column in the estuary of the river Quempillén (Ancud, Chile)

Source	df	MS	F	p
(A) Salinity				
Tidal cycle	12	163.4	46.61	*0.0001*
Season	3	1223.3	14.86	*0.0001*
Tidal cycle × season	36	56.6	16.15	*0.0001*
Error	288	3.5		
(B) TPM				
Tidal cycle	12	3.66	0.99	0.4584
Season	3	277.01	1.25	0.3261
Tidal cycle × season	36	5.24	1.41	0.0741
Error	192	3.69		
(C) OPM				
Tidal cycle	12	0.52	0.96	0.4865
Season	3	33.9	2.41	0.1039
Tidal cycle × season	36	0.53	0.96	0.5321
Error	192	0.54		

(A) Salinity, (B) total particulate matter (TPM) and (C) organic particulate matter (OPM). Data were analyzed by two-way repeated measures ANOVA, *p* values in italic indicate statistical significance

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Fig. 1 *Crepipatella dilatata*. Mean values (± SD) for environmental variables [Salinity, no units; *TPM* total particulate matter (mg L^{-1}); *POM* particulate organic matter (mg L^{-1})] in the water column during tidal cycles in the Quempillén estuary at different times of the year. **a** Summer (January); **b** autumn (May); **c** winter (August); **d** spring (November)

The interactions between tidal cycle and season and between all three main effects were also significant ($p < 0.0001$ and 0.0264 respectively, Table 2B). No tidal cycle effect was observed on the frequency of rasping the substrate in summer or autumn, although radular activity was much greater in non-brooding females than in brooders (Fig. 2c, f). In winter there was almost no scraping activity in the non-brooders except for a period of 3 h at low tide, when values increased sharply (Fig. 2i). The pattern was less clear in the brooding females, where radular activity was lower during the second half of the tidal cycle, after the tide turned. During spring, radular activity was lowest at high tide in brooders and non-brooders but increased as the tide fell before decreasing during the flood tide to reach initial values at high water (Fig. 2l). Values were higher in non-brooders than in brooders, except at high tide.

Radular activity in males
Mucous ball
There were significant differences among seasons in radular activity associated with removing mucous balls from the food pouch in males ($p = 0.0005$, Table 3A, Fig. 3a, d, g) and also in the interaction term between year and tidal cycle ($p = 0.0073$, Table 3A, Fig. 3a, d, g). However, for the factor tidal cycle alone no significant differences were observed ($p = 0.391$, Table 3A, Fig. 3a, d, g). The highest activity in mucous ball transfer occurred during spring (mean 14 extrusions h^{-1} ± SD 10), the lowest in winter ($3 ± 7$) (Fig. 3d).

Mucous cord
Significant differences were observed among seasons and tidal cycles in radular activity for the purpose of manipulating the mucous cord; the interaction term was also

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Table 2 *Crepipatella dilatata.* **Effects of the tidal cycle (12 h), reproductive status (brooding and non-brooding) and season (summer, autumn, winter and spring) on radular activity (extrusions h^{-1}) for (A) mucous ball removal, (B) transfer of mucous cord to the mouth and (C) substrate rasping in females**

Source	df	MS	F	p
(A) Mucous ball removal				
Tidal cycle	12	351.9	4.17	*0.0002*
Reproductive status	1	123.8	0.31	0.5758
Season	3	1304.2	3.33	*0.0253*
Season × reproductive status	3	598.3	1.52	0.2162
Tidal cycle × season	36	181.5	2.15	*0.0001*
Tidal cycle × reproductive status	12	90.1	1.06	0.3839
Tidal cycle × reproductive status × season	36	60.2	0.71	0.8945
Error	720	84.3		
(B) Transfer of mucous cord to mouth				
Tidal cycle	12	10,632	4.30	*0.0001*
Reproductive status	1	12,683	0.79	0.3770
Season	3	402,757	25.14	*0.0001*
Season × reproductive status	3	33,560	2.09	0.1102
Tidal cycle × season	36	6478	2.62	*0.0001*
Tidal cycle × reproductive status	12	2538	1.02	0.4208
Tidal cycle × reproductive status × season	36	2948	1.19	0.2050
Error	720	2470		
(C) Substrate rasping				
Tidal cycle	12	7723	2.76	*0.0011*
Reproductive status	1	514,833	38.23	*0.0001*
Season	3	38,232	2.83	*0.0453*
Season × reproductive status	3	15,797	1.17	0.3274
Tidal cycle × season	36	8342	2.98	*0.0001*
Tidal cycle × reproductive status	12	4838	1.73	0.0557
Tidal cycle × reproductive status × season	36	4258	1.52	*0.0264*
Error	720	2791		

Data were analyzed by three-way repeated measures ANOVA; *p* values in italic indicate statistical significance

significant ($p < 0.0001$, $p < 0.0001$ and $p < 0.0001$, respectively; Table 3B, Fig. 3b, e, h). In autumn and spring, radular activity directing the mucous cordon towards the mouth increased as the tide fell and remained high throughout the rest of the cycle (Fig. 3b, h, respectively). Mean values for radular activity in handling the mucous cord were lowest in winter and similar throughout the tidal cycle (Fig. 3e).

Rasping

Scraping the substrate was the dominant activity of the radula throughout the study (Table 3C, Fig. 3c, f, i). There were no significant differences in rasping activity through the tidal cycle or among seasons ($p = 0.3396$, $p = 0.0511$ respectively; Table 3C), although the interaction term was highly significant ($p = 0.0054$, Table 3C), During winter and especially spring (Fig. 3f, i respectively), but not in autumn (Fig. 3c), rasping activity in males increased from a minimum at high tide to a maximum at low tide, before decreasing as the tide fell.
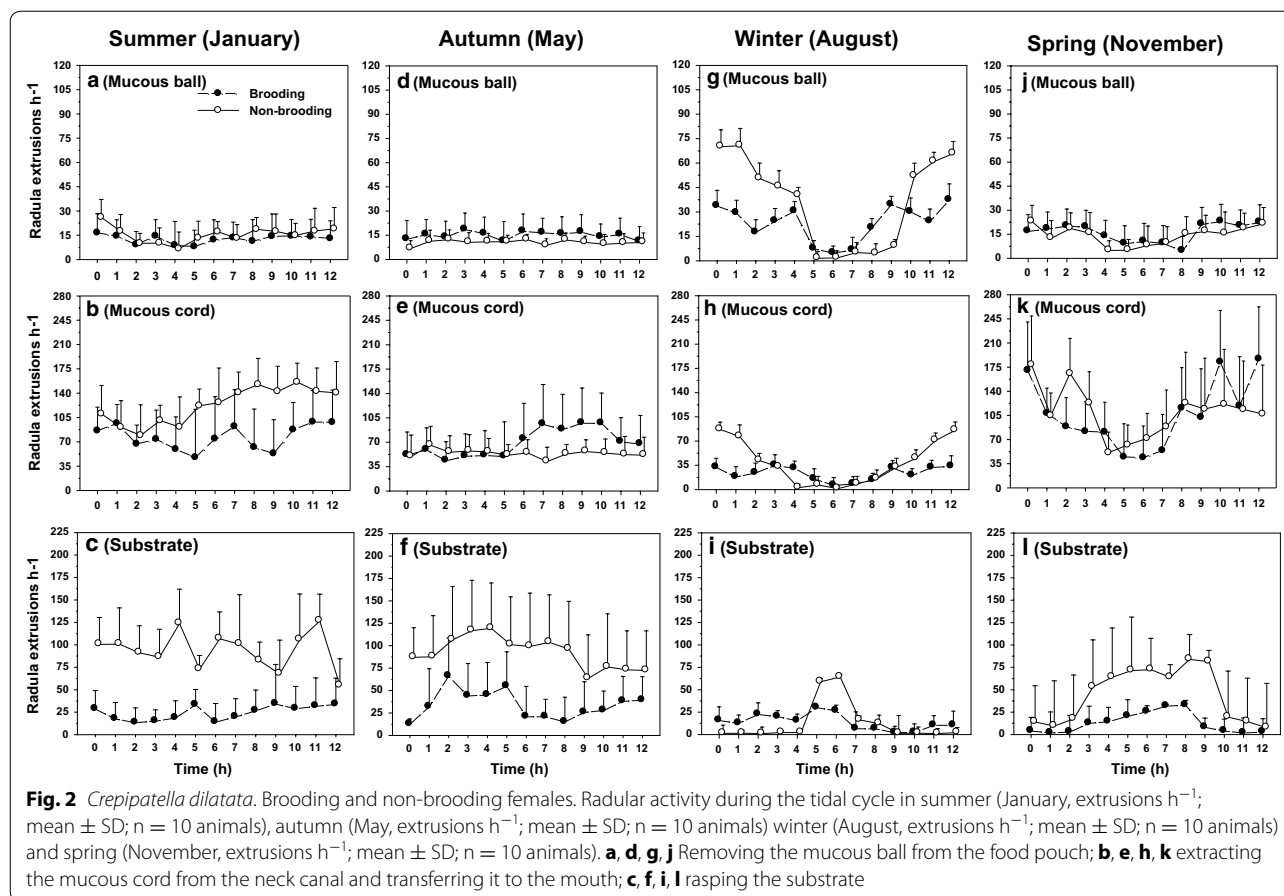
Relation between salinity and radular activity

In both incubating and non-incubating females radular activity ceased at salinities below 20 (Fig. 4a). Some activity was observed at salinities between 20 and 23, and at salinities greater than 23 values increased considerably. The same response to salinity was recorded in males, except that the critical salinity for radular activity was lower (19; Fig. 4b) than for females.

Discussion

Estuarine systems experience variations in environmental conditions in the water column as a result of stochastic atmospheric forcing [2, 37, 38] and tidal cycles [2, 8, 39]. In our study significant tidal and seasonal variation was observed in the concentration of seston (total particulate matter, TPM) in the Quempillén estuary, values being generally highest in summer (January), which is consistent with previous studies [37, 40, 41] and is typical of the seasonality characteristic of estuarine and shallow coastal systems in mid- to high latitudes [38, 41, 42]. The high TPM values we observed are associated with high standing stocks of phytoplankton in this estuary [7]. TPM values were low in winter (August), despite resuspension of sediment produced by strong wind events and precipitation [43]. As in nearby coastal areas [42], seston levels at Quempillén are largely determined by phytoplankton dynamics rather than sediment resuspension [7]. Similar patterns of variation (tidal superimposed on seasonal) in seston have also been recorded in coastal habitats, including estuaries, in other latitudes [2, 44, 39].

On several occasions we observed high variation in salinity associated with the tidal cycle in the Quempillén estuary, especially in winter and spring, when precipitation is high (http://164.77.222.61/climatologia/) and salinity can fall rapidly to values very close to zero at low tide during periods of heavy rainfall. Occasionally heavy rain can also occur in summer, but we did not encounter rainstorms during our January sampling. Salinity variation, particularly low salinity, is an important stress factor in estuarine systems [15, 16] and a significant regulator of physiological and behavioral processes in estuarine organisms [11, 13, 17]. The feeding behaviour of marine invertebrate suspension-feeders can be strongly impacted by exposure to low salinity, which frequently results in
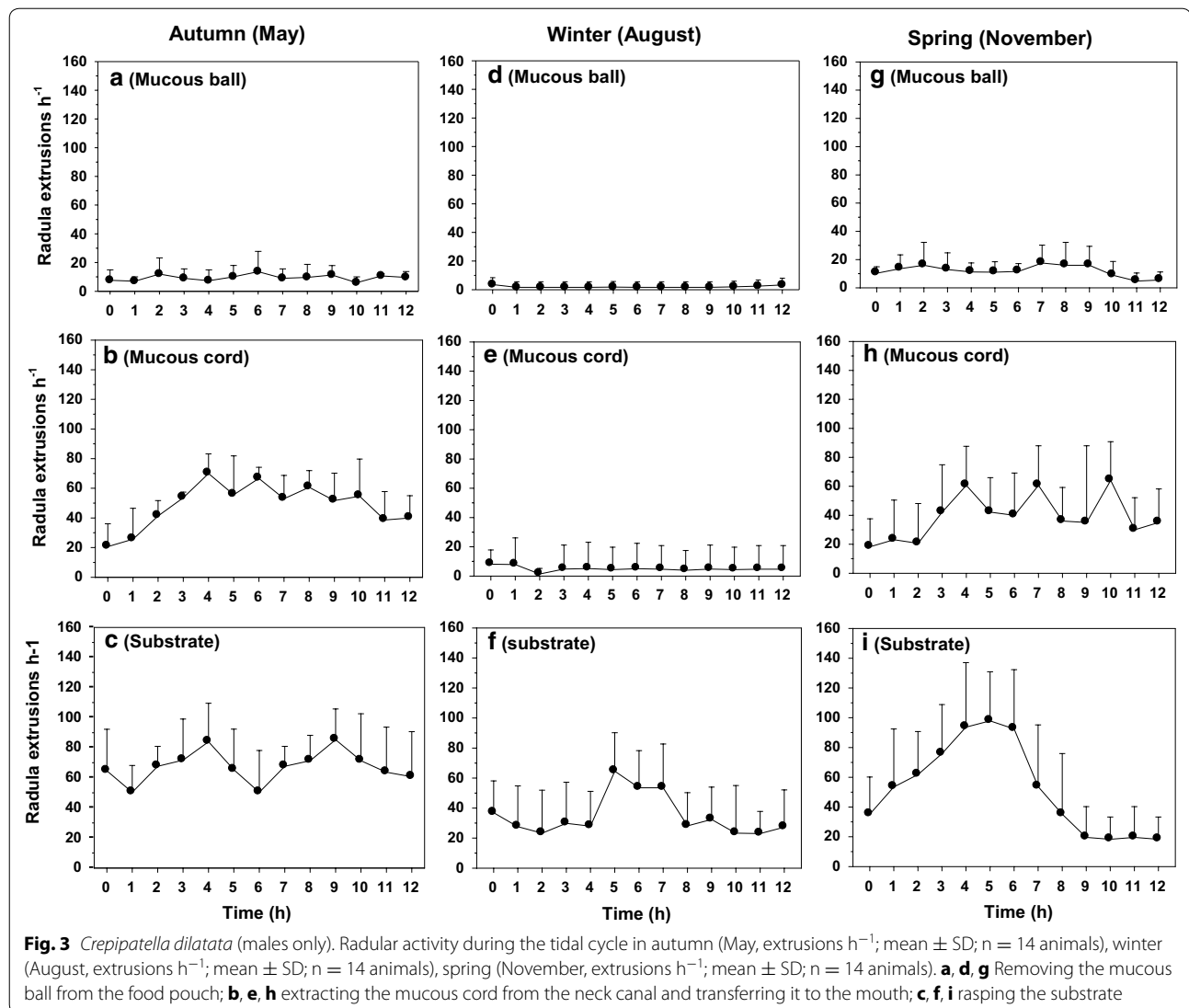
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Fig. 2 *Crepipatella dilatata*. Brooding and non-brooding females. Radular activity during the tidal cycle in summer (January, extrusions h^{-1}; mean ± SD; n = 10 animals), autumn (May, extrusions h^{-1}; mean ± SD; n = 10 animals) winter (August, extrusions h^{-1}; mean ± SD; n = 10 animals) and spring (November, extrusions h^{-1}; mean ± SD; n = 10 animals). **a**, **d**, **g**, **j** Removing the mucous ball from the food pouch; **b**, **e**, **h**, **k** extracting the mucous cord from the neck canal and transferring it to the mouth; **c**, **f**, **i**, **l** rasping the substrate

Table 3 *Crepipatella dilatata*. Effects of the tidal cycle (12 h) and season (autumn, winter and spring) on radular activity (extrusions h^{-1}) for (A) mucous ball removal, (B) transfer of mucous cord to the mouth and (C) substrate rasping in males

Source	df	MS	F	p
(A) Mucous ball removal				
Tidal cycle	12	39.10	1.06	0.3918
Season	2	4072.6	110.6	*0.0005*
Tidal cycle × season	24	70.24	1.90	*0.0073*
Error	275	36.8		
(B) Transfer of mucous cord to mouth				
Tidal cycle	12	3115.8	5.58	*0.0001*
Season	2	100,567.1	180.2	*0.0001*
Tidal cycle × season	24	2490.8	4.46	*0.0001*
Error	275	557.8		
(C) Substrate rasping				
Tidal cycle	12	2643.1	1.12	0.3396
Season	2	31,468.6	15.31	0.0511
Tidal cycle × season	24	4691.1	2.00	*0.0054*
Error	275	2346		

Data were analyzed by two-way repeated measures ANOVA; *p* values in italic indicate statistical significance

cessation of feeding or at least a reduction in clearance rate [1, 8, 11, 13, 17]. In many cases the individual also responds by partially or completely isolating itself from the surrounding environment to minimise osmatic stress [17]. Critical values of salinity have been identified below which calyptraeid gastropods adhere closely to the substrate, isolating the mantle cavity from the exterior, with the result that suspension-feeding ceases (e.g. salinity 22–24 for *C. peruviana*, 52; salinity 23–24 for *C. dilatata*, 17).

In our study, the degree to which the mantle cavity of *C. dilatata* was isolated from the ambient water, and the use of the radula in its various feeding modes, depended largely on environmental conditions, particularly salinity, and on the sex and reproductive status of the individual. In general, non-brooding females exhibited higher rates of radular extrusion for ingesting the mucous cord and for scraping the substrate than did brooders. In some suspension-feeding brooding mollusc species the incubated embryos, whether free or encapsulated, can remain for days or weeks within the mantle cavity of the female [23, 45, 46]. Their presence can physically interfere with the capture of food particles by the gill, thereby modifying
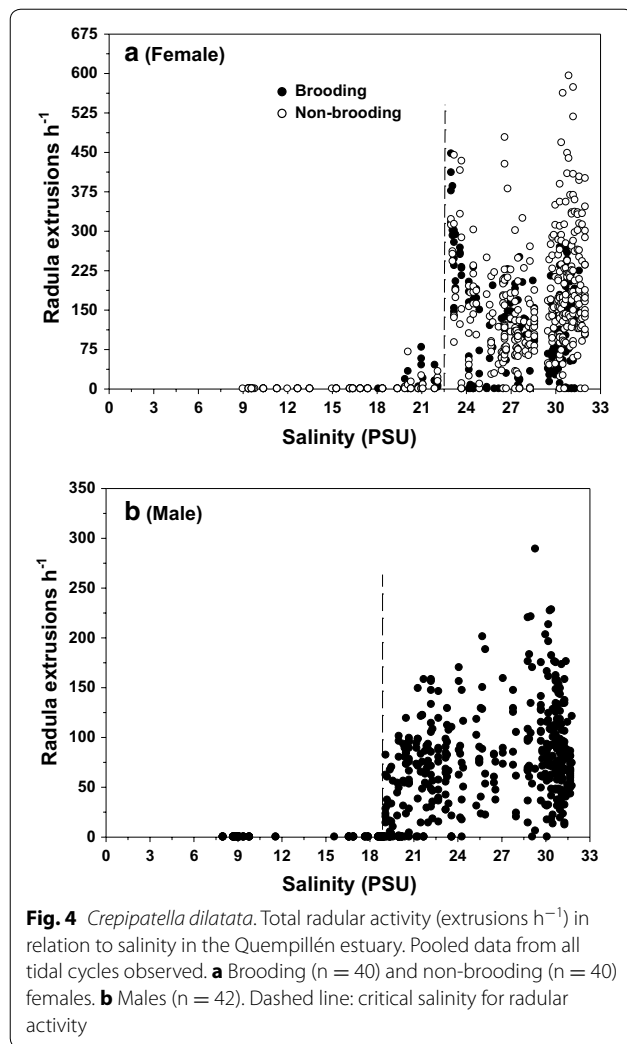
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Fig. 3 *Crepipatella dilatata* (males only). Radular activity during the tidal cycle in autumn (May, extrusions h^{-1}; mean ± SD; n = 14 animals), winter (August, extrusions h^{-1}; mean ± SD; n = 14 animals), spring (November, extrusions h^{-1}; mean ± SD; n = 14 animals). **a**, **d**, **g** Removing the mucous ball from the food pouch; **b**, **e**, **h** extracting the mucous cord from the neck canal and transferring it to the mouth; **c**, **f**, **i** rasping the substrate

clearance rate [47, 48]. In calyptraeids, particles captured on the gills are bound in mucous cords on the distal margins of the gill filaments and moved towards the mouth [23, 30]. Our observation that radular activity related to ingestion of the mucous cord is lower in brooding female *C. dilatata* than in non-brooders is consistent with a lower clearance rate. In some species, e.g. *C. peruviana*, the frequency of formation of mucous cords depends on the concentration of particles in the water column [36], as long as salinity exceeds the threshold level for isolation. Nevertheless, our data show that under the low salinity regimes prevailing in many of the tidal cycles we studied, salinity was a determinant of the amount of mucous cord material produced.

According to Navarro and Chaparro [25], removal of particles by the gill and their transfer to the mouth within a mucous cord located in a muscular canal on

the right side of the neck is the principal means of food acquisition by female calyptraeids, which are sessile. In our study of *C. dilatata* the rate of radular extrusion for ingesting the mucous cord varied seasonally and between brooding and non-brooding females. The lowest activity was recorded in the winter during periods in the tidal cycle when salinity decreased below 23. Under these conditions the mantle cavity is sealed from the external environment, suspension-feeding ceases and there is no production of a mucous cord. This behaviour has been recorded previously for *C. dilatata* by Montory et al. [49] and for *C. peruviana* and the oyster *Ostrea chilensis* by Chaparro et al. [17]. The difference in the critical salinity between females [23] and males [19] may be attributable to the fact that larval stages of marine invertebrates are often more sensitive than adults to low salinity [50–53].

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Fig. 4 *Crepipatella dilatata*. Total radular activity (extrusions h⁻¹) in relation to salinity in the Quempillén estuary. Pooled data from all tidal cycles observed. **a** Brooding (n = 40) and non-brooding (n = 40) females. **b** Males (n = 42). Dashed line: critical salinity for radular activity

The rate of radular extrusions for removing the mucous ball from the food pouch, however, did not differ between brooding and non-brooding females in our study. The origin of the material in the food pouch is not clear, although some comes from the anterior region of the gill and some from the mantle wall [30]. The mucous ball plays a much smaller role in feeding than the mucous cord, which carries far more material to the mouth; furthermore, material from the food pouch can be rejected as pseudofaeces as well as ingested, at least in *C. peruviana* [36]. Thus it is possible that the process of moving material to and from the food pouch can continue in brooding females despite the apparently reduced feeding activity.

In both brooding and non-brooding females the activity of the radula in removing the mucous ball from the food pouch was strongly influenced by the tidal cycle during winter, reaching minimum values at low tide.

Under these conditions salinity can decrease well below the critical value of 22–23 at which the female responds by isolating the mantle cavity from the external environment, thereby protecting the soft tissues and the embryos (in the case of brooders) from osmotic stress [17, 49, 50, 54]. Suspension-feeding ceases, and the very low rates of exclusion of the radula for removal of material from the food pouch may represent the arrival in the food pouch of residual particles in the mantle water and/or particles in the mantle tracts, or the use of material accumulated in the food pouch before isolation of the mantle cavity. In males there was no evidence that the tidal cycle affected radular activity in removing the mucous ball at any time of the year.

We observed differences among seasons and within tidal cycles in radular activity associated with scraping of the substrate in *C. dilatata*, as well as differences among males, brooding females and non-brooding females. Incubating females displayed much less scraping than non-brooders throughout the year. In *C. peruviana* the incubated capsules are affixed to the substrate and occupy as much as 87% of the area within the mantle cavity [55], restricting access to the substrate by the radula. Assuming that *C. dilatata* is similar in this respect, the substrate surface accessible for grazing is limited to the small area beneath the shell margin, reducing the availability of biofilm material for grazing. The fact that radular activity is reduced when the area available for grazing is limited may be a response by the individual to increase its efficiency of use of the food resource. Males exhibit a higher rate of radular activity in scraping throughout the year, including periods of low salinity, than in removing the mucous ball or ingesting the mucous cord. This does not necessarily imply that more energy is obtained through substrate grazing than suspension-feeding, since the relationship between the frequency of radular extrusion and food ingestion must vary among the different forms of feeding and according to environmental conditions. The data for rates of radular exclusion indicate the "division of labour" for the radula among three separate tasks associated with feeding, and inferences cannot be drawn about the nutritional significance of each type of radular activity under any given set of conditions.

In *C. dilatata*, juveniles and males are motile [35] and therefore have much more opportunity to complement suspension-feeding with substrate grazing [28]. Similar behaviour has been observed in motile individuals of *C. convexa* [56] and *C. adunca* [57]. During periods when the salinity is below the critical value of 19 and the *C. dilatata* male isolates the mantle cavity, preventing suspension-feeding, substrate rasping can continue, as demonstrated by a high rate of radular extrusion. When salinity increases and the mantle is re-irrigated,

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suspension-feeding resumes and the radula both scrapes the substrate and pulls the mucous cord into the mouth.

Throughout the year, about 65% of radular extrusions in the brooding female *C. dilatata* are for the purpose of ingesting the mucous cord, 18% for rasping the substrate and 15% for removing the mucous ball from the food pouch. In non-brooding females, mucous cord ingestion remains the principal activity of the radula (46% of total extrusions), but substrate rasping is relatively greater (40%) than in brooders and mucous ball removal accounts for a similar proportion (14%). Most of the radular activity in males (55%) was associated with substrate grazing, 12% with removing the mucous ball (similar to females) and 33% with manipulating the mucous cord. Thus, unlike males, females use the radula more for ingesting the mucous cord (i.e. suspension-feeding) than for substrate grazing. The feeding behaviour of both males and females is modulated by salinity, independently of the quality and quantity of seston in the estuary, but the principal determinants of radular activity are the mode of reproduction (brooding in females) and the ability to move (males). Calyptraeid gastropods are unusual in having two discrete feeding mechanisms, rasping the substrate with the radula and suspension-feeding with the gill.

In estuarine invertebrates, especially sessile suspension-feeders, salinity is the principal environmental factor to which feeding activity responds [8, 15, 17]. Our data for *C. dilatata* demonstrate differential responses in feeding behaviour in a sequential hermaphroditic species according not only to salinity but also to the degree of mobility of the individual (sessile vs. non-sessile), which is dependent on its sex and reproductive status. Incubation of embryos in calyptraeids results in modification of the feeding behaviour of the female, including restriction of the area available for rasping, as in *C. dilatata*, and changes in availability of suspended particles owing to changes in the inhalent flow associated with the presence of embryos, as in *C. fecunda* [33, 55].

Abbreviations
TPM: total particulate matter; POM: particulate organic matter.

Authors' contributions
ORC, CJS and VMC conducted all the surveys and the experiments. All the authors contributed to the design of research, analysis of results and subsequent writing of the manuscript. JAM conducted the statistical analyses. All authors read and approved the final manuscript.

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