Radular Force Performance of Stylommatophoran Gastropods (Mollusca) with Distinct Body Masses Reflect Diverging Feeding Patterns

WENCKE KRINGS (✉ wencke.krings@uni-hamburg.de)

Universität Hamburg

CHARLOTTE NEUMANN

Universität Hamburg

MARCO T. NEIBER

Universität Hamburg

ALEXANDER KOVALEV

Christian-Albrechts-Universität zu Kiel

STANISLAV GORB

Christian-Albrechts-Universität zu Kiel

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Abstract

The forces exerted by the animal's food processing structures are important parameters in studying trophic specializations to specific food spectra. Even though molluscs represent the second largest animal phylum, exhibiting an incredible biodiversity accompanied by the establishment of distinct ecological niches including the foraging on a variety of ingesta types, only few studies have been previously focused on the biomechanical performance of their feeding organs. To lay a keystone for future research in this direction, we investigated the *in vivo* forces exerted by the molluscan food gathering and processing structure, the radula, for five stylommatophoran species (Gastropoda). Radular forces were measured along two axes using force transducers which allowed us to correlate forces with the distinct phases of radular motion. The chosen species and individuals represent different body mass classes enabling the correction of forces measured for body mass. A radular force quotient, $AFQ = \text{force/bodymass}^{0.67}$, of 4.3 could be determined which can be used further for the prediction of forces generated in Gastropoda. Additionally, some specimens were dissected and the radular musculature mass as well as the radular mass and dimensions were documented. Our results clearly depict the positive correlation between body mass, radular musculature mass, and exerted force. Additionally, it was clearly observed that the radular motion phases, exerting the highest forces during feeding, changed with regard to the ingesta size: all smaller gastropods rather approached the food by a horizontal, sawing-like radular motion leading to the consumption of rather small food particles, whereas larger gastropods rather pulled the ingesta in vertical direction by radula and jaw resulting in the tearing of larger pieces.

Introduction

The typical force exerted by feeding organs is a useful parameter indicating specializations to distinct food types, as it correlates with the food spectrum [see e.g. 1–7; for a review for stress-related puncture mechanics, see 8]. This topic had been studied quite intensively in vertebrates [for a summary of the relevant literature, see 6]: bite force analyses had been performed on mammals [e.g. 9–13], reptiles [e.g. 14–17], fish [e.g. 18–20], and birds [e.g. 21–22]. Even though the majority of animal species belong to the invertebrates, unfortunately fewer work focused on the forces exerted by their structures involved in either gathering or acquiring food due to the difficulties of an experimental set-up for studies of small structures. Exceptions and pioneers in this field are studies performed on representatives of Arthropoda: spiders, crustaceans, scorpions, and insects [23–29].

For molluscs, even though they represent the second specious animal group [e.g. 30] with around 80,000 recent species only within the Gastropoda [31], only few studies approached the forces exerted by their feeding organ, the radula. Since the species belonging to this animal phylum occupy almost any marine, freshwater or terrestrial environment and established extremely varied ecological niches [e.g. 32], accompanied with feeding on a wide range of food sources with various mechanical properties, mollusc trophic specializations are of very high interest for the evolutionary biology of this group. Their radula, one important molluscan autapomorphy and being interface between the organism and its ingesta (food,

minerals), is highly diverse and offers an immense opportunity to study the structural adaptations enabling feeding on distinct ingesta types [see also 33].

The radula consists of a thin, chitinous membrane with rows of embedded, sometimes mineralized teeth which is supported by thick, underlying odontophoral cartilages and moved by numerous muscles of the buccal mass. The sometimes highly complex radular motion [see e.g. 34–39] brings the tooth cusps in contact with the ingesta leading to the tearing, cutting, and gathering of food.

As the teeth are involved as actual organism-ingesta interface, usually the shape of teeth or overall morphology of the radula had been examined and compared with the ingesta [e.g. 40–54; also examined for the tooth anchorage: 55]. In the context of phenotypic plasticity, different shapes of radular teeth as an answer to shifts of the ingesta have also been studied [45, 56–66]. Sometimes these analyses are complemented by material property estimations of teeth [38, 67–75]. Additionally, ingesta consumption, grazing activity, food choice experiments, and fecal analyses for diverse gastropod species have been investigated relating the gastropods with their preferred food, the abundance of food or other parameters of the microhabitat [76–82; for comprehensive reviews on diet of Heterobranchia, see 83–84].

The majority of previous studies have been focused on the radular teeth themselves, but the forces exerted by this organ or its biomechanical performance have unfortunately only been investigated in few papers devoted (1) to the feeding force calculations [85; or force calculations for radula-inspired gripping devices: 86], (2) to the experiments revealing the forces needed to remove algae [87–88], or (3) to the first *in vivo* experiment performed on a single mollusc species [89].

Here, to approach the topic of trophic specialization in molluscs and to lay a keystone for further studies, we investigated the *in vivo* radular forces, while foraging, for five different stylommatophoran species using force transducers following the protocol of [89]. These species were selected according to their similar radular type (isodont) and similar radular motion. As the species selected represent distinct body mass classes, the relationship between the body mass and radular force could be determined. We found that the stylommatophoran species perfectly follow the predictions for scaling of force and body mass as the radular force output scales to body mass^{0.67} with the quotient 4.25 and the Relative Force I scales to body mass^{-0.33} with the quotient 4.35. Additionally, we performed buccal mass dissections in some specimens, succeeded by the determination of the radular (buccal mass) musculature and radular mass. Our results clearly depict that the measured radular forces correlate with the body mass and with the radular musculature mass. However, when corrected for the body mass smaller individuals generated relatively stronger forces. Regardless the maximum values of exerted forces, all gastropods were able to feed on the same food type and size, but smaller gastropods, to cope with the offered food, used a different radular motion pattern than the larger ones, which is reflected by the radular force measurements and corresponding video footage.

Material And Methods

For force measurements we have chosen five stylommatophoran species (Gastropoda: Heterobranchia), because all possess a similar radular type (isodont), have a similar radular foraging motion [for details on motion and radular type see e.g. 39,89–95], and represent distinct body mass classes (Fig. 1): immature (one juvenile stage) and mature *Lissachatina fulica* (Bowdich, 1822), mature *Cepaea nemoralis* (Linnaeus, 1758), mature *Cepaea hortensis* (Müller, 1774), mature *Helix pomatia* Linnaeus, 1758, and mature *Arion vulgaris* Moquin-Tandon, 1855. Overall, 24 individuals were used for feeding experiments. Individuals of *L. fulica* were received from private animal breeders, the other species were collected in Hamburg, Germany, in May 2020. Species identification is based on the relevant literature.

The selected gastropods species were assigned to different body mass classes (large sized animals = mature *L. fulica* [N = 5 individuals], medium sized animals = *H. pomatia* and immature *L. fulica* [N = 10 individuals], small sized animals = *C. nemoralis*, *C. hortensis*, *A. vulgaris* [N = 9 individuals]) and cohorts (mature *L. fulica* [N = 5 individuals], immature *L. fulica* [N = 9 individuals], mature *H. pomatia* [N = 1 individual], mature *C. nemoralis* [N = 2 individuals], mature *C. hortensis* [N = 1 individual], mature *A. vulgaris* [N = 6 individuals]). Before each experiment, individuals were weighed (body mass with shell) (see Supplementary Table 1).

Forces exerted by the radula (= Absolute Forces) were measured following the protocol of [89]. Snails were placed on an acrylic platform with a small hole of 4 mm diameter. The platform was attached to a laboratory jack so that the height could be adjusted. Food stripes (sliced to pieces of 3 [width] x 2 [length] x 20 or 40 [height] mm; either carrot or fresh strawberry depending on the specific preference of the species) were glued to a needle, which was mounted onto a force transducer FORT-10 (World Precision Instruments, Sarasota, FL, USA) and stuck through the hole so that the snail could feed on it, but without the involvement of the foot (Fig. 2). A 1000 g sensor was used for mature *L. fulica*, for all other individuals a 25 g sensor was used. Forces could only be measured in either vertical or horizontal direction not in both simultaneously, thus the experimental set-up was remodeled to receive data for both directions [see also 89]. The force transducers were connected to an amplifier (Biopac System, Inc., CA, USA) and a computer-based data acquisition and processing system (AcqKnowledge™, Biopac Systems, Inc., v.3.7.0.0, World Precision Instruments, Sarasota, FL, USA). Not all force peaks were analyzed in AcqKnowledge™ due to the large sample size, but about 30 maximal and minimal force peaks were evaluated per feeding unit (for the detailed quantity of evaluated force peaks per species, individual, and direction see Supplementary Table 1), overall 4407. Forces were corrected for mean body mass with shell to receive Relative Force I (force/ mean body mass with shell, mN/ g). Mean and standard deviations were calculated and statistical analysis was performed with the program JMP® Pro 14 (SAS Institute Inc., Cary, NC, 1989–2007) comparing the exerted forces between (a) the distinct body mass classes, (b) the cohorts, (c) the directions, (d) the individual animals. A Shapiro-Wilk test, followed by a one-way ANOVA, and a Tukey-Kramer test with connecting letters report identifying homogenous groups was carried out. For linear regression of Absolute Force and Relative Force I *versus* whole body mass mean values were displayed on logarithmic axes with excel 2013 (Microsoft Corporation, Redmond, USA) and

trend lines were determined. A radular force quotient, $AFQ = \text{absolute force} / \text{body mass}^{0.67}$, and a relative force quotient, $RFQ = \text{relative force} / \text{body mass}^{-0.33}$, were determined.

Detailed radular motion, while feeding on a flat surface was documented with a Keyence VHX-500 digital microscope (KEYENCE, Neu-Isenburg, Germany) by placing the individual on an acrylic platform, providing flour paste as food [see also 39,89]. The behavior, while foraging on a carrot, was documented with an iPad Pro (11 Zoll; Apple Inc., Cupertino, USA) equipped with a 12 megapixel wide angle lens with 30 frames per second (see Supplementary Videos 1 and 2). Videos were cut and cropped with Adobe Premiere Pro 2020 (Adobe Inc., San Jose, USA).

Some animals (see Supplementary Fig. 8) were either killed by brief boiling (shelled animals) or by placing them in carbonated water (slugs). They were preserved in 70% EtOH and inventoried in the malacological collection of the Zoological Museum Hamburg (ZMH) of the Centrum für Naturkunde (CeNak); *Cepaea nemoralis*: ZMH 154748/8, *Helix pomatia*: ZMH 154749/1, *Lissachatina fulica*: ZMH 154751/2, *Cepaea hortensis*: ZMH 154754/1, *Arion vulgaris*: ZMH 154747/12.

The shells of dead specimens were removed and the soft parts were weighed to receive body mass without shell. Forces were corrected for body mass without shell to receive Relative Force II (force/ body mass without shell, mN/ g). To estimate the mass of the entire buccal mass (BRJ), the radula and jaw (RJ), and the buccal mass musculature (B) these specimens were dissected which was documented with a Keyence VHX-500 digital microscope (KEYENCE, Neu-Isenburg, Germany). The BRJ was first extracted, freed from surrounding tissue (see Supplementary Fig. 7) and weighed in wet condition with an accuracy weighing machine (Sartorius Cubis, MSE, Sartorius AG, Göttingen, Germany). Subsequently the radula and jaw (RJ) were separated from the buccal mass musculature (B) manually; RJ and B were weighed in wet condition. B and RJ were then dried for one week and weighed again to obtain data on dry mass. Forces were corrected for dry B to receive Relative Force III (force/ dry B mass, mN/mg) and for dry RJ to receive Relative Force IV (force/ dry RJ mass, mN/mg).

For scanning electron microscope (SEM) images radulae and jaws were rewetted and cleaned with proteinase K digesting food particles according to the protocol of [96], followed by a short ultrasonic bath. Structures were mounted on SEM stubs, coated with palladium and visualized with a Zeiss LEO 1525 (One Zeiss Drive, Thornwood, USA). Radular length, width, and area could be calculated. Forces were corrected for radular area to receive Relative Force V (force / radular area, mN/mm²).

Results

Radula and its teeth

All analyzed species possess an isodont radula (Supplementary Figs. 3–6). *Lissachatina fulica* displays ~ 84 teeth per row (no central tooth, 16 lateral and 26 marginal teeth on each side), *Helix pomatia* ~ 87 teeth per row (one central tooth, 23 lateral and 20 marginal teeth on each side), *Arion vulgaris* ~ 75 teeth

per row (one central tooth, 16 lateral and 21 marginal teeth on each side), *Cepaea nemoralis* and *C. hortensis* ~ 51 teeth per row (one central tooth, 12 lateral and 13 marginal teeth on each side). The jaws of all species are thick, curved, chitinous plates with ribs (i.e. odontognathous).

Radular motion

Video footage reveals the radular motion and feeding behaviour (see Supplementary Videos 1 and 2). While feeding, the radula is pushed simultaneously in ventral (vertical down) and anterior (horizontal anterior) direction, before the organ is finally pulled in dorsal (vertical up) and posterior (horizontal posterior) direction and the mouth is closed. With the first part of the motion the radula loosens food items from the ground and collects particles, which are transported into the mouth opening in the latter phase of the feeding action. When feeding on larger ingesta (e.g. a piece of carrot; see Supplementary Video 1), the anterior part of the radula and the jaw act in concert as counter bearing squeezing and pulling the ingesta. When comparing the feeding behaviours of different individuals we can see that the large sized individuals can completely enclose the carrot piece with their lips, resulting in a dragging on the carrot in vertical direction, tearing large pieces, whereas the small and medium sized individuals are not comfortable with this due to the small dimension of their mouth. These individuals usually nibble on edges of the item, cutting and slicing smaller pieces in rather horizontal direction employing their radula like a saw, sometimes involving additionally the foot as a clamp (Supplementary Video 2). All gastropods were able to consume the food items offered, but small sized individuals needed to invest approximately 800–900% and medium sized ones 400–500% more time to consume the similar sized food items than large sized gastropods.

Force measurements

Overall, 4407 individual force peaks were evaluated (for evaluated quantity of peaks per species, individual, and direction see Supplementary Table 1). For Absolute Forces exerted by the body mass classes see Fig. 3; for Absolute Forces exerted by the cohorts see Fig. 4 and Supplementary Table 2; for the Absolute Forces of individual gastropods see Supplementary Figs. 1, 2, and Supplementary Tables 3, 4.

Comparing the Absolute Forces regardless the direction between the three body mass classes of animals (Fig. 3A) we detect significant differences ($p < 0.0001$, F-ratio: 336.03, df: 2). Large sized individuals are capable of exerting highest forces (mean $\pm$ standard deviation; 73.96 ± 62.49 mN; $N = 1025$ evaluated force peaks), followed by the medium sized (41.44 ± 49.18 mN; $N = 2581$), and finally small sized individuals (14.46 ± 24.49 mN; $N = 801$). When comparing the Relative Force I regardless the direction between the three body mass classes of animals (Fig. 3B) we detect significant differences between groups ($p < 0.0001$; F-ratio: 346.10; df: 2). Here, small sized individuals exhibited the highest Relative Force I (6.01 ± 8.40 mN/g), followed by the medium sized (2.47 ± 2.94 mN/g), and finally large sized animals (0.84 ± 0.74 mN/g).

The Absolute Force (regardless the direction) quotient, $AFQ = \text{absolute force} / \text{body mass}^{0.67}$, is found to be 4.25 and the Relative Force I (regardless the direction) quotient, $RFQ = \text{relative force} / \text{body mass}^{-0.33}$, 4.35 (see Fig. 5).

Absolut Forces assorted to direction (Fig. 3C) also differed significantly between body mass classes (for horizontal anterior: $p < 0.0001$, F-ratio: 240.09, df: 2; horizontal posterior: $p < 0.0001$, F-ratio: 104.69, df: 2; vertical down: $p < 0.0001$, F-ratio: 393.05, df: 2; vertical up: $p < 0.0001$, F-ratio: 544.38, df: 2). For horizontal anterior direction, the highest forces were, however, exerted by the medium sized class (-82.22 ± 55.10 mN; $N = 617$), followed by the large (-26.97 ± 2.91 mN; $N = 223$), and finally small sized gastropods (-15.34 ± 7.87 mN; $N = 180$). For horizontal posterior direction, the highest forces were again exerted by the medium sized class (74.52 ± 49.76 mN; $N = 536$), followed by the small sized gastropods (39.56 ± 35.37 mN; $N = 211$), and finally large (38.82 ± 19.49 mN; $N = 323$). In the direction vertical down, the large sized gastropods showed highest forces (-22.77 ± 17.79 mN; $N = 107$), followed by the medium (-3.94 ± 2.30 mN; $N = 440$), and finally small gastropods (-0.86 ± 0.44 mN; $N = 210$). The same is found for vertical up: large gastropods (147.35 ± 41.35 mN; $N = 372$), medium (14.72 ± 10.40 mN; $N = 988$), and finally small ones (1.48 ± 0.72 mN; $N = 200$). When comparing the Relative Force I assorted to direction (Fig. 3D) we again detect significant differences between body mass classes (for horizontal anterior: $p < 0.0001$, F-ratio: 181.52, df: 2; horizontal posterior: $p < 0.0001$, F-ratio: 620.28, df: 2; vertical down: $p < 0.0001$, F-ratio: 109.90, df: 2; vertical up: $p < 0.0001$, F-ratio: 233.52, df: 2). Here, for horizontal anterior direction, the highest Relative Force I was exerted by the small sized gastropods (9.26 ± 9.08 mN/g), followed by the medium (4.98 ± 3.59 mN/g), and finally large ones (0.30 ± 0.13 mN/g). For horizontal posterior direction, the highest Relative Force I was again exerted by the small (13.63 ± 8.67 mN/g), followed by the medium (4.28 ± 2.65 mN/g), and finally large sized class individuals (0.43 ± 0.20 mN/g). In the direction vertical down, the small sized gastropods showed highest Relative Force I (0.50 ± 0.28 mN/g), followed by the large (0.25 ± 0.21 mN/g), and finally medium gastropods (0.24 ± 0.17 mN/g). For vertical up direction, the large gastropods (1.69 ± 0.54 mN/g) exerted highest Relative Force I, followed by the medium (0.92 ± 0.65 mN/g), and finally small ones (0.85 ± 0.51 mN/g).

When comparing Absolut Forces regardless the direction between cohorts (Fig. 4A) we found significant differences ($p < 0.0001$, F-ratio: 114.24, df: 5). Highest forces were exerted by mature *L. fulica* (73.96 ± 62.49 mN; $N = 1025$), followed by *H. pomatia* (55.98 ± 65.25 mN; $N = 270$), immature *L. fulica* (39.73 ± 46.67 mN; $N = 2311$), *C. nemoralis* (23.01 ± 35.08 mN; $N = 316$), *A. vulgaris* (10.25 ± 11.79 mN; $N = 325$), and finally *C. hortensis* (6.10 ± 6.60 N = 160). When comparing Relative Force I regardless the direction between cohorts (Fig. 4B) we found significant differences ($p < 0.0001$, F-ratio: 198.21, df: 5). Highest Relative Force I was exerted by *C. hortensis* (9.43 ± 10.17 mN/g), followed by *C. nemoralis* (6.85 ± 9.99 mN/g), *A. vulgaris* (3.52 ± 3.72 mN/g), immature *L. fulica* (2.59 ± 3.03 mN/g), *H. pomatia* (1.47 ± 1.72 mN/g), and finally mature *L. fulica* (0.84 ± 0.74 mN/g).

Comparing the Absolut Forces assorted to direction between cohorts (Fig. 4B) we found significant differences (for horizontal anterior: $p < 0.0001$, F-ratio: 96.17, df: 5; horizontal posterior: $p < 0.0001$, F-ratio: 82.07, df: 5; vertical down: $p < 0.0001$, F-ratio: 156.98, df: 5; vertical up: $p < 0.0001$, F-ratio: 2176.63, df: 5).

For horizontal anterior direction, the highest forces were exerted by *H. pomatia*, followed by the immature *L. fulica*, mature *L. fulica*, *A. vulgaris*, *C. hortensis*, and finally *C. nemoralis* (for values and connecting letters see Supplementary Table 2). For horizontal posterior direction, the highest forces were exerted by *H. pomatia*, followed by *C. nemoralis*, the immature *L. fulica*, mature *L. fulica*, *A. vulgaris*, and finally *C. hortensis*. For vertical down direction, the highest forces were exerted by mature *L. fulica*, followed by *H. pomatia*, the immature *L. fulica*, *A. vulgaris*, *C. nemoralis*, and finally *C. hortensis*. For vertical up direction, the highest forces were exerted by mature *L. fulica*, followed by the immature *L. fulica*, *H. pomatia*, *C. nemoralis*, *A. vulgaris*, and finally *C. hortensis*. When comparing the Relative Force I assorted to direction between cohorts (Fig. 4C) we found significant differences (for horizontal anterior: $p < 0.0001$, F-ratio: 428.67, df: 5; horizontal posterior: $p < 0.0001$, F-ratio: 728.85, df: 5; vertical down: $p < 0.0001$, F-ratio: 139.78, df: 5; vertical up: $p < 0.0001$, F-ratio: 135.26, df: 5). For horizontal anterior, the highest Relative Force I was exerted by *C. hortensis*, followed by *A. vulgaris*, the immature *L. fulica*, *C. nemoralis*, *H. pomatia*, and finally mature *L. fulica* (for values and connecting letters see Supplementary Table 2). For horizontal posterior direction, the highest Relative Force I was exerted by *C. nemoralis*, followed by *C. hortensis*, *A. vulgaris*, the immature *L. fulica*, *H. pomatia*, and finally mature *L. fulica*. For vertical down direction, the highest Relative Force I was exerted by *C. hortensis*, followed by *A. vulgaris*, the immature *L. fulica*, mature *L. fulica*, *C. nemoralis*, and finally *H. pomatia*. For vertical up direction, the highest Relative Force I was exerted by mature *L. fulica*, followed by *C. hortensis*, the immature *L. fulica*, *A. vulgaris*, *C. nemoralis*, and finally *H. pomatia*.

Masses of body, radula, buccal mass musculature, and radular sizes

Highest whole body mass (see Supplementary Table 1) was measured for *L. fulica* mature 1, followed by *H. pomatia*, *L. fulica* immature 9, *C. nemoralis* 1, *A. vulgaris* 2, 4, 5, *C. nemoralis* 2, *A. vulgaris* 1, and finally *C. hortensis*.

Overall, we found that the body mass (with and without shell) relates in proportion to the masses of the whole buccal mass (wet; BRJ), the radular musculature (buccal mass musculature, wet and dry; B), and the radula and jaw (wet and dry; RJ). When individuals were heavier, they usually possessed higher muscle mass, a heavier radula and jaw (see Supplementary Fig. 8 and Supplementary Table 6). Exceptions were: *L. fulica* immature 9 (18 g body mass and 205.01 mg whole buccal mass) and *H. pomatia* (38 g body mass and 163.30 mg whole buccal mass), *C. nemoralis* 1 (3.60 g body mass and 27.20 mg whole buccal mass), and *A. vulgaris* 2 (3.50 g body mass and 56.66 mg whole buccal mass). Comparing mature (mature 1: 78.00 g body mass and 286.80 mg BRJ) and immature *L. fulica* (immature 9: 18.00 g body mass and 205.01 mg BRJ) we found that the body mass increases for the factor ~ 4 and BRJ increases for the factor 1.4.

We found that smaller gastropods are mostly capable of exerting higher forces per radular muscle mass (see Supplementary Fig. 9 and Supplementary Table 6). *Cepaea hortensis* exerted the highest force per radular musculature mass, followed by *C. nemoralis* 1, *A. vulgaris* 4 and 5, *C. nemoralis* 2, *H. pomatia*, *L.*

fulica mature 1, *L. fulica* immature 9, and finally *A. vulgaris* 1 and 2. The same sequence was also found for force per body mass without shell. With the exception of *A. vulgaris* 4 and 5, exerting the highest forces per radula and jaw mass, we detected the same order for this parameter.

The radular length and width (see Supplementary Fig. 8 and Supplementary Table 5) do not consistently correlate with body mass, the highest radular width was measured for *L. fulica* mature 1 (width in μm : 4933; length in μm : 5178), followed by *A. vulgaris* 5 (4725; 2156) *L. fulica* immature 9 (3945; 8165), *H. pomatia* (3023; 9077), *A. vulgaris* 4 (2597; 4947) *A. vulgaris* 1 (1999; 3765), *A. vulgaris* 2 (1869; 5701), *C. nemoralis* 2 (1780; 3492), *C. nemoralis* 1 (1558; 4906), and finally *C. hortensis* (1034; 4351). Both gastropods with the largest width possessed shorter radulae than all other specimens. *L. fulica* immature 9 possessed the largest radular area (32.21 mm^2), followed by *H. pomatia* (27.44 mm^2), *L. fulica* mature 1 (25.54 mm^2), *A. vulgaris* 4 (12.85 mm^2), *A. vulgaris* 2 (10.66 mm^2), *A. vulgaris* 5 (10.19 mm^2), *C. nemoralis* 1 (7.64 mm^2), *A. vulgaris* 1 (7.52 mm^2), *C. nemoralis* 2 (6.22 mm^2), and finally *C. hortensis* (4.50 mm^2). The radular area again did not consistently correlate with the whole body mass.

Discussion

Force output is often referred to as proportional to muscle mass^{0.67}, the muscle cross-sectional area [e.g. 97–98], or to body mass^{0.67} [e.g. 99] whereas the forces, corrected for body mass (force/mass), are referred to be proportional to body mass^{-0.33} [e.g. 99]. The stylommatophoran species examined here perfectly follow the predictions for scaling of force and body mass. Following previous studies [27, 100] we here experimentally determined a radular force quotient AFQ, $\text{AFQ} = \text{force}/\text{bodymass}^{0.67}$, of 4.25 and a relative force quotient RFQ, $\text{RFQ} = \text{relativeforce}/\text{bodymass}^{-0.33}$ of 4.35 which can be further used for predictions of forces in Gastropoda.

The here measured absolute feeding forces for *Helix pomatia* are in a similar range as to those documented by [89] for *Cornu aspersum*, both gastropod groups have comparable body mass. We detected that gastropods with a higher body mass and a larger body size were capable of exerting higher radular forces, which is not surprising. However, the relationship between forces generated by the feeding organ and body size is well documented for vertebrates, but not for molluscs. When forces are corrected for body size or mass, ecological adaptations related with this parameter became more pronounced [e.g. 4–5, 9, 12, 21; for hypotheses about body size related evolution of bite force, see also 101–102]. When determining the radular force per body mass (termed Relative Force I), we see that smaller gastropods were capable of exerting the highest forces, followed by the medium, and finally large ones, similar to e.g. Carnivora [12]. This is also not surprising, because of different scaling of body mass and cross-sectional area of muscles [103–104]. Usually, specimens exhibiting a higher body mass also possess a proportionally higher mass of buccal mass [correlation between buccal mass size and gastropod body size was also previously described by 56]. However, the immature and adult *Lissachatina fulica*, exhibiting strong differences in body mass and size, but having almost similar buccal mass sizes and masses, are an exception to this rule. In most experiments mature *L. fulica* exerted higher radular forces

than the immature gastropods, but some measurements (outliers) revealed that immature *L. fulica* are also capable of generating the same feeding forces as the mature ones. This could be an indication that the ingesta type does not change during ontogeny; however, this aspect awaits further investigation. However, our analyses of Relative Force I (Fig. 4) reveal distinct radular force patterns for cohorts, both *Cepaea* species have a wide range of exerted forces, followed by *Arion*, *Lissachatina* immature, and finally, with the smallest range, *Lissachatina* mature. This could be an indication that *Cepaea* potentially feeds naturally on a broader food spectrum whereas *Lissachatina* is more restricted, but this also awaits further investigations.

In past studies, it has been shown that the forces generated usually correlate with the muscle mass, muscle size, or muscle diameter [for vertebrates: e.g. 13,21,106; for invertebrates: 107]. This is congruent to the here observed patterns in gastropods. However, for precise interpretation of the relationship between buccal mass size and the mass of the radular muscles (buccal mass musculature) precise knowledge about the functional role of each feeding muscle is important, which is not the case for these taxa studied here. There are some detailed studies analyzing the *in vivo* buccal mass movement and buccal mass muscle function in *Aplysia* [91–92, 94, 108]. However as *Aplysia* belongs to the Opisthobranchia and is not closely related to the taxa studied here the function of the buccal mass muscles for the stylommatophoran taxa studied here cannot be assigned yet. However, to deeply understand the relationship between measured radular forces involved in specific radular motions, the function of each muscle, their work in concert, as well as the muscle and fiber size, length, or diameter need to be investigated. As there are many different parameters that relate with feeding force [for vertebrates: e.g. the skull geometry and size: 4,15,22,109–111; or muscular development: 106], it is very difficult to produce reliable models for molluscs due to the lack of solid experimental data. Additionally, studies on invertebrates reveal that muscle stress varies considerably depending on the muscle [112–114]. This could also be the case for molluscs as the forces exerted per radular muscular mass differ extremely between the analyzed stylommatophoran individuals (see Supplementary Fig. 9 and Supplementary Table 6). This again shows that pure anatomy-based studies on the muscle systems of invertebrates do not necessarily provide data on physiology [see also 29].

Our results clearly indicate that larger and heavier animals exerted higher forces in the vertical directions, whereas medium and small individuals exerted higher forces in horizontal directions. This is additionally supported by the analyses of the video footage showing that larger animals rather pull with radula and jaw, whereas smaller individuals use their radular often like a saw in anterior-posterior direction when approaching the food item (see Supplementary Video 1 and 2). This shift in feeding behavior seems to depend on the ingesta size in relation to the mouth opening size. Smaller and medium sized gastropods - even though capable of embracing the whole item with the lip - seem to prefer this alternative feeding pattern. An ingesta-depending shift in feeding pattern, i.e. dynamics of swallowing, had been documented for other gastropod taxa when altering parameters of the ingesta [108], e.g. its hardness [115], its load and width [116], or its size [117–119]. Additionally, gastropods of different sizes have been found to feed on different ingesta types, possibly correlating with ontogenetic size changes of the mouthparts [120].

As already stated above, heavier gastropods were usually capable of exerting higher radular forces than smaller ones (except for immature and adult *L. fulica*). This indicates that larger gastropods might forage on a broader spectrum of ingesta, as it had been previously described for turtles [110]. However, the smaller individuals were able to exert higher forces per body mass and additionally show a distinct radular motion pattern resulting in a distinct feeding pattern. All experiments resulted in the consumption of the ingesta offered, but smaller gastropods invested more time. This effect had also been observed for lizards [3] and should be further investigated for Mollusca. Thus, we can conclude that in studies of feeding forces of gastropods with a similar radular type, teeth, and radular motion, adaptations to ingesta might only been detected by studying feeding efficiency and invested time. We hope that in the future more biomechanical, physiological and functional morphological studies will approach the topic of trophic specialization in molluscs using feeding force experiments on molluscs possessing different radular types and feeding on more distinct ingesta types.

Declarations

Authors' contribution

WK and SG initiated the project, discussed the data analysed, performed statistical analyses. CN performed experiments, analysed data, and contributed to earlier versions of the manuscript. SG provided experimental design and contributed to the biomechanical aspects of the manuscript. MTN performed species identification. AK contributed to data analysis. All authors contributed to and approved the final manuscript for publication.

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Figures

Figure 1

Mollusc species used in this study. A. Mature *Lissachatina fulica*. B. *Helix pomatia*. C. Immature *Lissachatina fulica*. D. *Arion vulgaris*. E. *Cepaea nemoralis*. Scale bar = 4 cm.

Figure 2

Experimental set-up [modified from 89] and characteristic radular force measurements. A. Gastropods were placed on an acrylic platform with a hole of 2 mm diameter. The sliced food (e.g. carrot), glued to a needle, was firmly mounted to a force transducer connected with an amplifier and computer-based data acquisition and processing system. The food was stuck through the hole, so that animals could feed on it without involving their foot. B. Image of the mouth opening taken through the glass platform. C. Characteristic radular force measurement curves, mN, of mature *Lissachatina fulica*, (above) and *Arion vulgaris* (below); left side: vertical direction (positive peaks = pulling up, negative peaks = pushing down), right side: horizontal direction (positive peaks = posterior direction, negative peaks = anterior direction). JA = jaw, FL = flange, FO = foot, LI = lip, RA = radula.

Figure 3

Absolute Force and Relative Force I for distinct body mass classes (large-, medium-, and small-sized gastropods). A. Absolute Force (regardless to the direction of measurement). B. Relative Force I (regardless to the direction of measurement). C. Absolute Force assorted to directions. D. Relative Force I assorted to directions. Letters are connecting letters from the Tukey-Kramer test and identify homogenous groups (letters at same height depicts which groups are statistically compared).

Figure 5

Linear regression, displayed on logarithmic axes, with trend line of A. Absolute Force mean versus whole body mass mean. A radular force quotient, $AFQ = \text{absolute force} / \text{body mass}^{0.67}$, of 4.25 was determined; and B. Relative Force I mean versus whole body mass mean. A relative force quotient, $RFQ = \text{relative force} / \text{body mass}^{0.33}$, of 4.35 was determined.

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