



PREDATION BY *DUGESIA POLYCHROA* (PLATYHELMINTHES: TRICLADIDA) PREVENTS THE ESTABLISHMENT OF *PHYSA ACUTA* (GASTROPODA: PULMONATA) IN HARD-SUBSTRATUM HABITATS

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ABSTRACT: Despite half a century of research on planarian-gastropod interactions, the population-level impact of planarian predation on littoral pulmonates is not well known. We have quantified the predation of the common lacustrine planaria *Dugesia polychroa* Schmidt on the ubiquitous snail *Physa acuta* Drap., a potential keystone grazer in benthic littoral communities, in a high-replicate laboratory experiment. Planarian predation pressure was maintained constant, and experimental populations of *P. acuta* were size-structured simulating young-rich small groups (re)colonising the habitat (e.g. after release from predation). *D. polychroa* caused a quick (week 2) reduction in the density of hatchlings and juveniles, followed by significant reduction in the numbers of young (at the onset of reproductive age) and larger, reproductive-age adults (week 3). Planarians did not have any effect on the size of reproductive-age large adults, but oviposition decreased significantly in week 2, and all *P. acuta* populations in planarian-present aquaria went extinct within six weeks. Dugesiid-induced extinction mainly occurred by precluding population turnover. The results strongly suggest that active predation by *D. polychroa* on *P. acuta* can be high under some environmental conditions, effectively preventing the (re)establishment of small, colonising populations of *P. acuta* in hard-substratum habitats.

KEY WORDS: gastropods, planarians, interspecific interactions, predation-induced behaviour, population regulation, lake littoral, benthic communities

The ubiquitous freshwater pulmonate *Physa acuta* Draparnaud, 1805 is a formidable grazer on detritus and periphyton, while live macrophytes are seldom eaten (SHELDON 1987, LOMBARDO & COOKE 2002). *P. acuta* and the closely related *P. fontinalis* enhance macrophyte growth even at high nutrient concentrations (JONES et al. 1999, LOMBARDO 2001), which would otherwise favour periphytic overgrowth at the expense of macrophyte well-being (MOELLER et al. 1988), potentially boosting littoral phytoplankton biomass and leading to undesirable decreases in water clarity (SCHEFFER 1998). Its commonness (e.g. DILLON 2006) and high grazing abilities have led LOMBARDO (2001) to postulate a keystone role for *P.*

acuta in macrophyte-based littoral benthic communities, also supporting the use of benthic food-web biomanipulation as a tool to maintain or restore high water clarity in productive shallow lakes (BRÖNMARK & WEISNER 1992, LOMBARDO 2001, 2005). Based on abundant empirical evidence (e.g. HOSPER 1997 and references therein), such biomanipulation emphasises the reduction of benthivorous fish to release snails from predation and (re)boost their densities (e.g. BRÖNMARK & WEISNER 1992). However, the possible negative effects of small-bodied invertebrate predators on *P. acuta* and their potential cascading effects on littoral water quality remain much less known.

Dugesiid planarias include aquatic snails in their diet (REYNOLDS & YOUNG 1963, GEE & YOUNG 1993, TRIPET & PERRIN 1994, SEABY et al. 1995). However, despite the abundant literature on gastropod-planarian interactions, the regulation of snail populations by planarias remains poorly understood. Major discrepancies lay in laboratory vs. field studies, with the former pointing to high predation pressure on snails (e.g. TRIPET & PERRIN 1994) and the latter reporting on coexisting abundant planarian and snail populations (e.g. PYEFINCH 1937, MACAN 1977). MACAN (1977) explained the conundrum of natural high-density snail-planarian assemblages with low predatory efficiency by planarias coupled with their ability to withstand long periods of starvation. This argument is partly supported by laboratory findings of predominant scavenging on damaged or recently dead "prey" and/or low affinity for live prey by dugesiid planarias (JENNINGS 1957, SEABY et al. 1995). However, some direct evidence of dugesiid predation on live snails exists (REYNOLDS & YOUNG 1963, TRIPET & PERRIN 1994, LOMBARDO et al. unpublished).

While the operculum of prosobranch snails appears to be an effective defense against intrusive predators such as leeches (e.g. BRÖNMARK & MALMQVIST 1986, KELLY & CORY 1987), pulmonates like *P. acuta* may be less protected against such predators. *P. acuta* has a thin, globose shell whose mouth is larger than the underlying foot, potentially exposing the soft parts to intrusive predators. *P. acuta* relies heavily on behavioural defences such as shell shaking, fleeing above the water surface, and/or abrupt detachment from the substratum and quick deployment to the water surface thanks to the air bubble under its mantle (WILKEN & APPLETON 1991, MCCOLLUM et al. 1998, TURNER et al. 1999, authors' personal observations). Such behaviours are typically effective against leeches (BRÖNMARK & MALMQVIST 1986, WILKEN & APPLETON 1991), but their effectiveness against planarias remains poorly known.

In a detailed laboratory study with *Dugesia lugubris* Schmidt, 1861, TRIPET & PERRIN (1994) estimated

that on average 2.77 individuals of *P. acuta* were eaten in each 48-h, multiple-choice trial, a result in apparent stark contrast with LOMBARDO et al.'s (unpublished) findings of very low individual predatory rates by the closely related *Dugesia polychroa* Schmidt, 1861 on live pulmonates. Both studies were carried out in the laboratory using small hunting arenas (dishes with diameter of ~7–10 cm), with estimations of predatory rates extracted from experimental designs that targeted specific aspects of dugesiid predation on *P. acuta*, namely the effect of shell shape on susceptibility to predation in TRIPET & PERRIN (1994) and predator preference among snails at different stages in their life cycles in LOMBARDO et al. (unpublished). Furthermore, in a rare direct comparison of parallel field and laboratory studies, KESLER & MUNNS (1989) found much higher losses to predatory insects in the small-setting laboratory experiments, and warned that such high rates were likely overestimates due to low spatial heterogeneity and unnaturally high snail density in the experimental aquaria.

We have quantified the impact of common *D. polychroa* planarias on small, size-structured experimental populations of *P. acuta*, simulating small natural populations recovering after reductions in benthivorous fish following a hypothetical benthic food-web biomanipulation. The experiment was conducted indoors using small aquaria (~4 litres) to provide conditions closer to natural than in typical Petri-dish laboratory investigations of planarian-gastropod interactions (REYNOLDS & YOUNG 1963, TRIPET & PERRIN 1994, LOMBARDO et al. unpublished). Planarian predation pressure was maintained constant at two medium-sized adult individuals per aquarium, while the experimental population structure of *P. acuta* was initially biased in favour of juvenile individuals. The snail populations were monitored weekly in planarian-present aquaria vs. planarian-absent controls until the last treatment replicate populations went extinct after six weeks.

MATERIAL AND METHODS

STUDY ORGANISMS

P. acuta is a North American species long naturalised in Europe (TAYLOR 2003, GARCÍA-BERTHOU et al. 2007). It is very common in permanent, productive lakes throughout western and Mediterranean Europe, including Italy (GIROD et al. 1980, GARCÍA-BERTHOU et al. 2007), where it was introduced in the 1860s (CIANFANELLI et al. 2007). *P. acuta* is still expanding eastward in Europe (MICHALIK-KUCHARZ 2008), and has been introduced as far as South Africa (WILKEN & APPLETON 1991) and Australia (KEFFORD et al. 2003). Also

following the recent species consolidation within the group (DILLON et al. 2002, WETHINGTON & LYDEARD 2007), *P. acuta* is considered one of the most ubiquitous aquatic macroinvertebrates in the world (DILLON 2006). *P. acuta* inhabits all types of freshwater ecosystems, including those with moderately high salinity (KEFFORD et al. 2003), and is particularly tolerant of thermal and organic pollution (GIROD et al. 1980, COSTIL & CLEMENT 1996, DILLON 2006, authors' personal observations). *P. acuta* is a generalist herbivorous scraper, feeding on periphyton and decaying plant material and other detritus (REAVELL 1980, LOMBARDO



2001). Living macrophyte tissue is eaten only when preferred food is not available (SHELDON 1987, LOMBARDO & COOKE 2002). Species identification was based on morphological traits of live individuals using GIROD et al. (1980) and GLÖER & MEIER-BROOK (2003). Nomenclature follows DILLON et al. (2002), WETHINGTON & LYDEARD (2007), and BROWN & LYDEARD (2010).

D. polychroa is common in warm, productive lakes throughout Italy and much of Europe (BALL & REYNOLDS 1981, YOUNG & REYNOLDS 1999, PONGRATZ et al. 2003, NORENA JANSSEN 2011); it has been introduced in North America in the late 1960s (BODDINGTON & METTRICK 1974), but it does not seem to have spread much from the original area of introduction in southeastern Canada, consistent with its poor dispersal abilities (PONGRATZ et al. 2003, VERBERCK et al. 2008). *D. polychroa* is a wide trophic generalist that includes gastropods in its diet (e.g. REYNOLDS & YOUNG 1963, BODDINGTON & METTRICK 1974), and may additionally act as a scavenger on damaged or recently dead prey (SEABY et al. 1995). Species identification was based on morphological traits of free and squash-mounted live individuals using BALL & REYNOLDS (1981). Nomenclature follows BALL & REYNOLDS (1981) and NORENA JANSSEN (2011).

Experimental animals were randomly picked from predator-free cultures maintained in a well-lit indoor laboratory at the Department of Environmental Sciences of the University of L'Aquila, and comprising individuals collected in the spring 2008 (*D. polychroa*) or starting from October 2007 (*P. acuta*). Culturing conditions were semi-natural, including natural (diffuse) sunlight and photoperiod and seasonal temperature, with short-term fluctuations mitigated by the indoor location. *D. polychroa* was originally collected at an open-canopy, shallow (~0.5 m), vegetation-devoid, gravel-bottom site (42°32'N, 12°44'E; WGS 84) along the northern shore near the western tip of Lake Piediluco. *D. polychroa* numerically dominated in a triclade-rich benthic community that also comprised *D. lugubris*, *Polycelis tenuis* Ijima, 1884, and *Dendrocoelum lacteum* O. F. Müller, 1774. The prosobranch *Bithynia leachii* Sheppard, 1823 dominated the rich in situ gastropod fauna, that also included numerically rare pulmonate species (*Planorbis planorbis* Linnaeus, 1758, *Radix auricularia* Linnaeus, 1758, and *Stagnicola fuscus* C. Pfeiffer, 1821). *P. acuta* was not found in Lake Piediluco, and was collected at a similar site in nearby Lake Ventina, where it coexisted with the introduced triclade *Dugesia tigrina* Girard, 1850. The two source lakes are hard-water, meso-eutrophic, seemingly hydrologically connected, and are located in central Italy within the River Tiber watershed (GAINO et al. 2001). Planarias and snails were fed ad libitum with natural organic material from the collection sites, integrated with lettuce and commercially available fish food in flakes or pellets. Material from differ-

ent lakes was kept in separate aquaria. Cultured populations remained abundant and healthy with sustained reproduction through and beyond the experiment period. Collection sites and culturing conditions are described in detail in LOMBARDO et al. (2011a, b).

EXPERIMENTAL SETUP

The experiment was carried out alongside the culturing aquaria, with animals being thus subjected to the same ambient conditions, including natural sunlight and photoperiod. Effects of *D. polychroa* on *P. acuta* were determined as differences between planarian-present treatment aquaria and planarian-devoid controls. Experimental aquaria consisted of small, clear-plastic boxes (23.5×15×14 cm) with ~4.25 l of tap water (water depth ~12 cm). Two small, rough-surface, clean cobbles were placed at the distant ends of each aquarium bottom to offer refuges and resting places for planarias and/or snails. A ~12–15 cm sprig of the periphyton-covered, finely dissected macrophyte *Myriophyllum spicatum* L., collected in situ in Lake Sinizzo (42°17'N, 13°33'E; ~15 km ESE of the city of L'Aquila in the River Aterno watershed) ~24–48 h before commencement of the experiment, also was placed in each aquarium to provide oxygen, food (biofilm and senescent plant parts) and refuge for the snails, as the macrophyte semi-firm texture was assumed to be disliked by *D. polychroa* (KIPP 2007, authors' personal observations). Macrophyte biomass placed in each aquarium was estimated volumetrically with a narrow-opening graduated cylinder. Tap water was set aside for the previous ~36–48 h to reach ambient temperature and lose excess chlorine.

Twenty four such aquaria, equally divided between 12 planarian-present treatments and 12 planarian-absent controls, were placed near a large W-facing window in a 3×8 grid organised in four contiguous blocks of six aquaria each, equally divided between treatments and controls, to distribute the unavoidable gradient in light conditions between treatment and control aquaria. Treatments and controls were assigned randomly within each block.

Two midsize adult planarias (total body length of individuals actively gliding in Petri dishes on 0.5 mm grid paper = 9–10 mm) were placed in each treatment aquarium on 11 June 2008 (t_0). Planarias were not subjected to a pre-experiment period of starvation, as any few-days starvation period was considered negligible with respect to the expected experiment duration. *P. acuta* individuals randomly picked from the culturing aquaria were assigned to three discrete age (size) categories: hatchlings/juveniles (shell height <3 mm), young adults at the onset of reproductive age (3–5 mm), and reproductive-age adults (>5 mm). Reproductive patterns were based on direct observations and published information (PERRIN 1986, CROWL &

COVICH 1990). Size categories were determined as shell height/length (measured along the shell main axis) of individuals roaming or resting in Petri dishes on a 0.5 mm grid paper for the smallest individuals, or with an electronic precision calliper (instrument error = 0.01 mm) for the larger adults. Two *P. acuta* individuals per size category were placed in each aquarium at t_0 . Given the paucity of published population-level studies of dugesiid-physid interactions under controlled conditions and an apparent natural mutual exclusion of *D. polychroa* and *P. acuta* in central Italian lakes (MASTRANTUONO & MANCINELLI 2005, authors' personal observations), the initial snail and planarian numbers were assigned semi-arbitrarily, with the purpose of providing a small, "colonising" group of snails and non-negligible yet detectable medium-term predation pressure by planarians. Aquaria thus prepared were left undisturbed until the first census.

Water was replaced and snails were counted in each aquarium every week. Water temperature, pH, and dissolved oxygen concentrations were determined in each aquarium before each weekly routine with a multi-parameter electronic probe. Equipment failure precluded some determinations in week 5 (wk₅); however, no obvious qualitative differences in aquarium conditions were observed between aquaria and with contiguous weeks. Aquaria were drained to the last ~2 cm of water, snails were counted, and the "spent" macrophyte sprigs were removed along with the bulk of the wastes. Once counted, the snails were placed back in the aquaria after water was refreshed with tap water set aside for the previous 36–48 h. Dead planarians (if any) were replaced with similar-sized individuals from the culturing aquaria, and any planarian individual in excess (i.e. produced through fission) was removed to maintain a constant predation pressure across treatment aquaria and in time. New, freshly field-collected *M. spicatum* sprigs were also placed in the refreshed aquaria. Aquaria thus refreshed were left undisturbed until the next weekly census. Egg clutches were not removed during the weekly routines, and the number of newly laid clutches was determined as differences between weekly counts.

The weekly determined snail variables included the number of live individuals in the three size categories, the number of newly laid egg clutches, and the shell height of live and dead large adults. As not all empty hatchling shells could be accounted for, net changes in snail densities were determined as weekly differences in the number of live individuals. New hatchlings/juveniles were added in small numbers at the end of the first three weekly censuses (6, 6, and 4 individuals per aquarium, respectively) to provide a small reservoir of freshly hatched individuals, simulating expanding populations in an active recruitment period. The number of hatchling/juvenile individuals added at the end of the first three weekly censuses was based on qualitative observations of general

adult-to-juvenile ratios in the culturing aquaria at that moment. Snail counts were expressed as numbers of individuals per aquarium. Though a per snail basis would have been more sensible biologically, the per aquarium basis was adopted also for egg clutches to circumvent the uncertainty derived from the unknown number of sexually mature "female" individuals at any given moment, especially for the age class of young adults, and to standardise statistical procedures and significance levels across snail variables.

An open-end approach was adopted. Aquaria where the snail population went extinct were cleaned and set aside, and counts of zero for all snail size categories were assigned, thus maintaining the balanced 12+12 design until the end of the experiment. Shell height of live or dead large adults was averaged over total counts within each experimental condition. The experiment was terminated once all 12 snail populations in one of the conditions (the treatment) went extinct, at the end of wk₆ on 25 July 2008.

STATISTICAL ANALYSIS

Differences in physico-chemical (e.g. water temperature) and snail variables (e.g. number of individuals) were tested with repeated-measures ANOVAs, simultaneously testing for between-condition differences and temporal changes. Physico-chemical variables were tested also by block. Between-block and treatment-vs.-control average values were additionally compared within each weekly sampling using a Student-Newman-Keuls (SNK) test [equivalent to a one-way ANOVA computed against the model $y = \text{average}(y)$ with $df=1,22$ for treatment-vs.-control comparisons, the default method used by our software]. Repeated-measures ANOVAs could not be applied to snail shell height data because of uneven and sometimes small sample sizes; analysis of such data remained limited to weekly treatment-vs.-control comparisons by means of two-tailed t-tests. Differences and/or temporal changes were considered significant for $p \leq 0.05$. Count-based data (i.e. number of snail individuals) were square-root-transformed (Anscombe formula: $x' = \sqrt{x + 3/8}$) and all other data were log-transformed [Bartlett's formula: $x' = \log_{10}(x + 1)$] before use in statistical procedures (ZAR 2009). Statistical analysis was performed using Addinsoft™ XLSTAT 2011.1.05. Background data for initial conditions were determined at one randomly chosen aquarium per block, and were not included in the ANOVA tests. Numerical pyramids, with the treatment and control *P. acuta* populations averaged over the 12 replicates, also were constructed for each weekly aquarium-based census, to provide straightforward snapshots of population structure. Due to the descriptive, albeit quantitative, nature of such data, numerical pyramids were not treated statistically.

INITIAL AND GENERAL EXPERIMENTAL CONDITIONS

Block-based initial conditions suggested seasonable temperature ($19.2 \pm 0.1^\circ\text{C}$; average \pm SE, $n=4$), circum-neutral pH (7.4 ± 0.1 ; average \pm SE, $n=4$), and high oxygen availability ($9.4 \pm 0.1 \text{ mg l}^{-1}$ and $101.0 \pm 1.3\%$ saturation of dissolved O_2 (DO) concentration; average \pm SE, $n=4$ for each). Water temperature determined before each weekly sampling routine remained in the $\sim 18.1\text{--}22.7^\circ\text{C}$ range, pH remained in the slight alkaline range ($\sim 6.7\text{--}8.6$), and dissolved oxygen concentration was always $\geq 7.6 \text{ mg l}^{-1}$ and at near- to super-saturation levels (Appendix Table A1). Midday irradiance, determined during randomly chosen sunny days with an aerial PAR-measuring light meter (instrument error $<1\%$), remained in the $\sim 10\text{--}25 \mu\text{mol m}^{-2} \text{ s}^{-1}$ range, simulating shaded conditions in shallow littoral zones (LOMBARDO 1995). Treatment and control aquaria received similar initial macrophyte biovolume (two-tailed t-test on log-transformed

data: $t=0.444$, $df=n_{\text{treatment}}+n_{\text{control}}-2=22$, $p=0.66$). Macrophyte material was later added according to general snail density in each aquarium, in order to minimise or delay limiting conditions for snails regarding this spatio-trophic resource.

As expected from the indoor light gradient from the aquaria placed closest to farthest from the window, background conditions varied significantly by block (Appendix Table A2). Physico-chemical background conditions also varied in time, as expected from the semi-natural experimental setting (water temperature), and/or from the adaptively changed macrophyte sprig biovolume (e.g. the temporal increase in DO was likely due to the increased macrophyte sprig biovolume) (Appendix Table A1). Despite the temporal and block-based variability, background conditions remained similar for treatment and control aquaria, with no factor interaction with time (Appendix Tables A3 and A4).

RESULTS

Temporal changes in the number of *P. acuta* hatchlings/juveniles, young adults, reproductive-age adults, and newly laid egg clutches were significant (Table 1). The number of hatchling and juvenile snails became significantly lower in *D. polychroa*-present aquaria by wk₂, while treatment-vs.-control differences for young and large adults became significant one week later (Fig. 1, Table 2). Snail density in control aquaria appeared to reach stable level by wk₄, though reproductive output continued to decrease until wk₅. Parallel decreases in reproductive output in control and treatment aquaria led to insignificant differences in the number of newly laid egg clutches in wk₃ through wk₅, but oviposition apparently resumed in control aquaria in wk₆ (Fig. 1). Oviposition in treatment aquaria stopped after wk₄.

Numerical pyramids also pointed to stable, young-rich *P. acuta* populations in control aquaria from wk₄ onward (Fig. 2). The age-class of young

adults gradually expanded in control aquaria, but shrank quickly in treatment aquaria, to become extinct in wk₅ (Fig. 2). Disappearance of juveniles and large adults one week later sanctioned the extinction of all *P. acuta* populations subjected to *D. polychroa* predation.

Body size of large, reproductive-age adults progressively decreased in all aquaria, leading to non-significant differences between predation-devoid controls and planarian-present treatment aquaria (Fig. 3, Table 3). Shell height of reproductive-age adults at time of death was somewhat variable, and treatment-vs.-control differences remained insignificant whenever computable (Fig. 3, Table 3).

Planarian predation was witnessed in a few instances. Planarias moving around the aquarium and encountering a (small) snail engulfed the shell with their body, while maintaining a firm grip on the smooth-plastic aquarium bottom or walls or the sub-

Table 1. Repeated-measures-ANOVAs for the hatchlings+juveniles (Juv, shell length $<3 \text{ mm}$), young adults (Ad1, shell length = $3\text{--}5 \text{ mm}$), large adults (Ad2, shell length $>5 \text{ mm}$), and newly laid egg clutches (Egg) in Fig. 1. Tests were performed on square-root-transformed data. For all ANOVAs, $df_{\text{experimental condition}}=1$, $df_{\text{time}}=5$, $df_{\text{condition} \times \text{time interaction}}=5$, between-subject $df_{\text{error}}=22$, and within-subject $df_{\text{error}}=110$

		Juv	Ad1	Ad2	Egg
experimental condition	<i>F</i>	132.849	88.645	44.971	25.264
	<i>p</i>	<0.0001	<0.0001	<0.0001	<0.0001
time	<i>F</i>	2.747	5.168	10.960	43.953
	<i>p</i>	0.021	0.0002	<0.0001	<0.0001
condition \times time interaction	<i>F</i>	24.479	28.966	14.439	1.649
	<i>p</i>	<0.0001	<0.0001	<0.0001	0.151

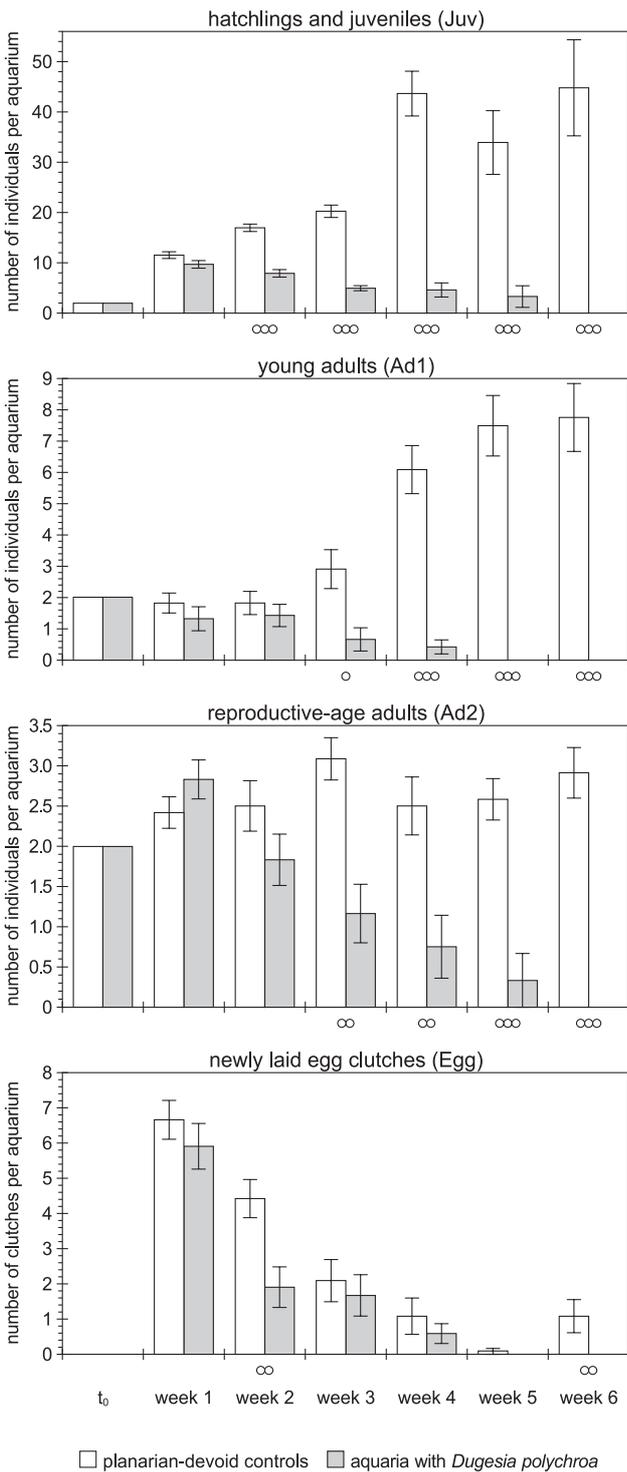


Fig. 1. Number of hatchlings and juveniles (Juv: shell height <3 mm), young adults at the onset of reproduction (Ad1: shell height = 3–5 mm), reproductive-age adults (Ad2: shell height >5 mm), and newly laid egg clutches (Egg) of *P. acuta* per aquarium during the 6-week experiment (average ±SE, n=12 for all). Differences between planarian-absent controls and planarian-present treatments, determined with repeated-measures ANOVAs that included weekly SNK-based treatment-vs.-control comparisons, are given at three levels of significance (O – $p \leq 0.05$, OO – $p \leq 0.01$, OOO – $p \leq 0.0001$). Detailed statistical results are in Tables 1 and 2

Table 2. Results of the SNK-based treatment-vs.-control comparisons [equivalent to a one-way ANOVA computed against the model $y = \text{average}(y)$ with $df=1,22$] for each weekly sampling within the repeated-measures-ANOVAs of Table 1, for the hatchlings+juveniles (Juv, shell length <3 mm), young adults (Ad1, shell length = 3–5 mm), large adults (Ad2, shell length >5 mm), and newly laid egg clutches (Egg) in Fig. 1. Significant treatment-vs.-control for $p \leq 0.05$

	Juv	Ad1	Ad2	Egg
week 1	0.065	0.206	0.203	0.348
week 2	<0.0001	0.430	0.146	0.004
week 3	<0.0001	0.002	0.0004	0.594
week 4	<0.0001	<0.0001	0.002	0.423
week 5	<0.0001	<0.0001	<0.0001	0.328
week 6	<0.0001	<0.0001	<0.0001	0.021

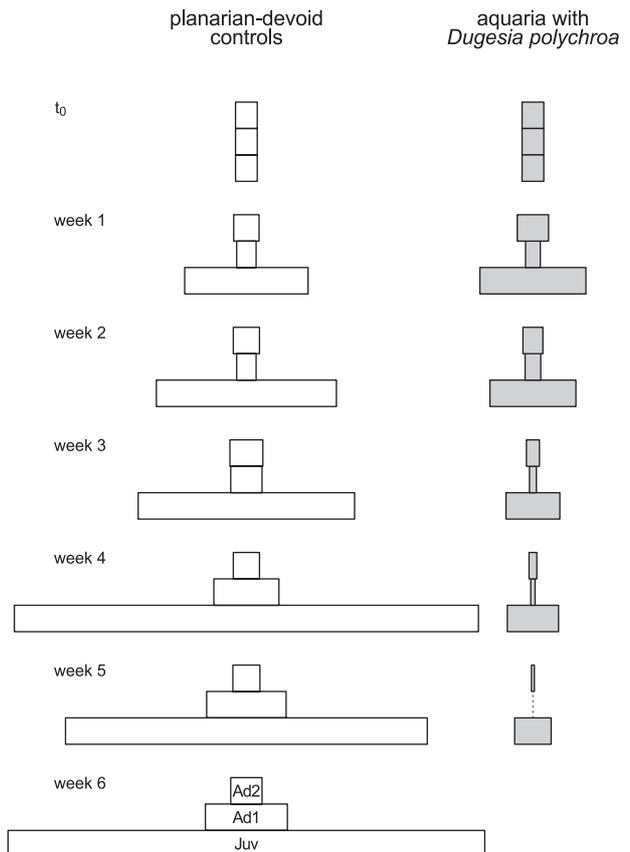


Fig. 2. Numerical pyramids describing the control and treatment *P. acuta* populations during the 6-week experiment, using the average values reported in Fig. 1. Juv – hatchling and juvenile individuals (shell height <3 mm), Ad1 – young adults at the onset of reproduction (shell height = 3–5 mm), and Ad2 – reproductive-age adults (shell height >5 mm). *P. acuta* went extinct in all 12 treatment aquaria by week 6

merged stones, immobilising the snail. The shell was then turned upside down, while the planaria inserted its ventral pharynx into the shell opening, and started to digest the snail's body externally (Fig. 4). The entire body was extracted from the shell in a few minutes. Prey capture was either very quick and successful, or the attacked snail managed to escape un-

harmd within a few seconds; prolonged struggles were never observed. Planarias did not appear to actively chase prey; encounters seemed to have been largely random and casual. *D. polychroa* captured and consumed the prey individually; the gregarious behaviour commonly displayed by other dugesiids (e.g. *D. tigrina*: CASH et al. 1993, authors' personal observation) was never observed. Planarias were seldom observed, and attacks were never observed, on the *M. spicatum* sprig. The few surviving *P. acuta* individuals in planarian-present aquaria were typically found on the macrophyte sprig during wk₄ and onward.

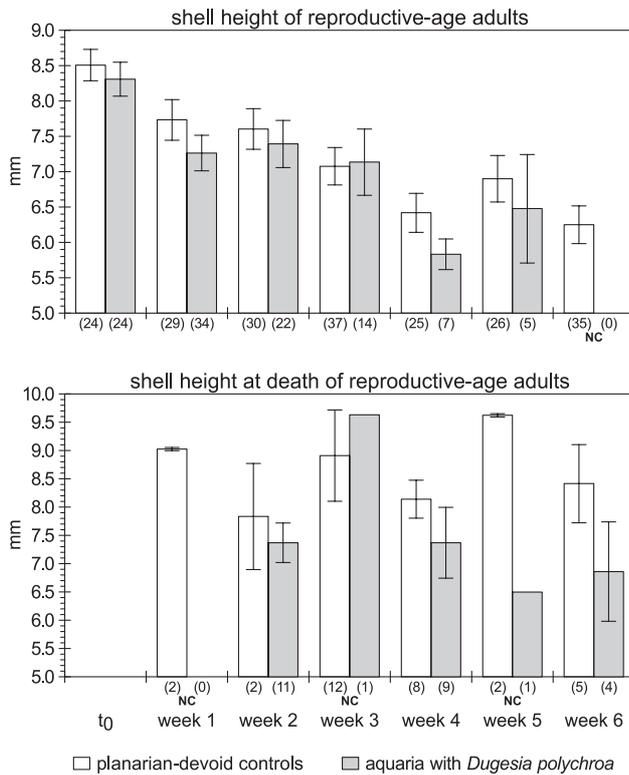


Fig. 3. Shell height of live and dead reproductive-age *P. acuta* adults during the 6-week experiment (average \pm SE, n as given at the base of each bar). Differences between planarian-absent controls and planarian-present treatments, determined with weekly two-tailed t-tests, were either not significant or not computable (NC: n_{ctrl} and/or $n_{\text{treat}} < 2$). Detailed statistical results are in Table 3

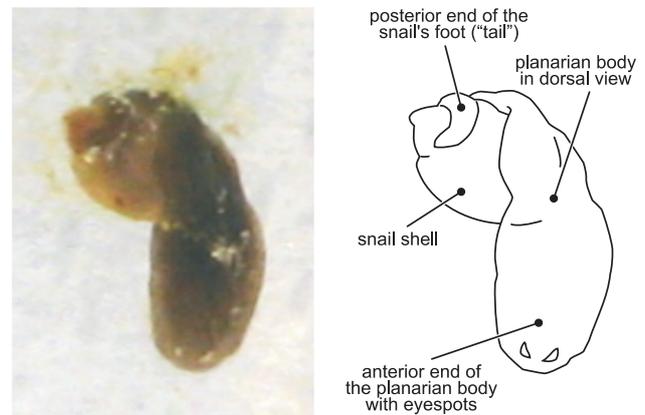


Fig. 4. A *D. polychroa* individual feeding on a subdued young adult (shell height = 3–5 mm) of *P. acuta*, as seen from above, during the experiment (left: picture; right: re-traced drawing). The shell is kept upside down by the posterior half of the planarian body, while the anterior half adheres tightly to the aquarium bottom. The planarian eyespots are visible as depigmented areas along the head margin. The background in the picture is white filter paper placed under the clear-plastic aquarium. The picture is un-manipulated except for cropping of excess background area and slight a posteriori brightness and contrast whole-picture digital adjustment to enhance clarity

Table 3. Results of the two-tailed t-tests comparing average shell height for live large adult snails and for large adults at death in treatment-vs.-control aquaria, displayed graphically in Fig. 3; blanks represent not computable tests (n_{ctrl} and/or $n_{\text{treat}} < 2$). Negative t values indicate control averages > treatment averages. Data were pooled by experimental condition (i.e. n = sum of all aquaria in each experimental condition within each week)

	shell height of									
	live large adult snails					large adults at death				
	n_{ctrl}	n_{treat}	t	df	p	n_{ctrl}	n_{treat}	t	Df	p
t ₀	24	24	0.944	46	0.350					
week 1	29	34	-1.167	61	0.248					
week 2	30	22	-0.486	50	0.629	2	11	-0.509	11	0.621
week 3	51	14	0.058	63	0.954					
week 4	33	6	-0.058	37	0.954	8	9	-1.212	15	0.244
week 5	31	4	0.541	33	0.592					
week 6						5	4	-1.462	7	0.187

DISCUSSION

The negative effect of *D. polychroa* on *P. acuta* was unequivocal (Figs 1 and 2). Direct observations of planarias capturing and consuming small prey (juveniles and, less commonly, young adults; Fig. 4) strongly suggest that the almost-immediate significant decrease in the number of juvenile snails was due to active predation. The observed mode of *D. polychroa* predation on *P. acuta* was similar to that described by KOPOWITZ (1970) for large-bodied, South African marine flatworms preying on limpets on sandy beaches. Similar predatory methods for such geographically and size/habitat-wise distant taxa suggest that free-living, predatory planarias share behavioural traits in addition to morphological characteristics, consistent with the relatively low placement in the evolutionary hierarchy of the phylum Platyhelminthes. Failure to account for all the juveniles (alive + empty shells) in planaria-present aquaria during the weekly censuses however suggests that at least some of the smallest snails were ingested whole, including the shell. Apparently “casual” encounters and the absence of active prey chase suggest that snails may be an occasional prey, supporting the view that dugesiid planarias are passive scavengers rather than active predators (JENNINGS 1957, SEABY et al. 1995), and that, when actively preying, preference is for small prey, with snails probably serving as a “refuge” prey to relieve interspecific competition in species-rich triclad communities (e.g., REYNOLDS & YOUNG 1963).

Absence of further changes in the number and relative abundance of the three snail age/size classes from wk₄ onward (Figs 1 and 2) suggests that control aquaria had reached carrying capacity by that time, despite the progressively increased macrophyte sprig biovolume. Such an interpretation is supported by the temporal variation in the number of non-predatory casualties among large adults in control aquaria (n values in the bottom panel of Fig. 3), displaying a peak during wk₃ and wk₄ and a return to low numbers afterward. Resumed oviposition in wk₆ also supports a return to an equilibrium once carrying capacity was reached after the initial density overshooting peak in wk₃-wk₄. Stabilised control populations however remained young-rich, and the casualties among the largest (=oldest) size/age classes were counterbalanced by new hatchlings (Fig. 2) despite a decline in reproductive output (Fig. 1), thus allowing population turnover.

The slightly delayed decline in the number of reproductive-age adult snails was preceded by a sudden crash in reproductive output in the presence of *D. polychroa* (Fig. 1), suggesting that planarian effects on larger snails may have been a combination of direct, active predation (especially when “preferred” smaller

individuals began to be scarce) and an indirect inhibition of foraging and reproductive activity. The much quicker decline in younger (shell height = 3–5 mm) than larger (shell height >5 mm) *P. acuta* adults aligns with *D. polychroa*'s “preference” for smaller prey and suggests that young adults were probably subjected to both direct predation (Fig. 4) and indirect effects on behaviour (decreased activity). Indirect effects may have been relatively more important for the larger adults, whose average size remained the same in planarian-present and control aquaria (Fig. 3). A scavenging action of planarias on weak or moribund large snails was also possible (SEABY et al. 1995).

Though direct evidence for planaria-snail interactions is not available, our hypothesis is supported by earlier observations of behavioural modifications (especially the inhibition of foraging activities) for *Physa heterostropha* (= *P. acuta*; DILLON et al. 2002) in the presence of molluscivorous sunfish (MCCOLLUM et al. 1998), and for other North American physids in the presence of fish and/or crayfish predators (ALEXANDER & COVICH 1991a, b, TURNER et al. 1999). The sudden, albeit temporary, crash in reproductive output in our planarian-present *P. acuta* populations may be due, additionally or alternatively, to adult snails holding oviposition while diverting resources to growth, in an attempt to reach a size refuge. Such a hypothesis is supported by the ability of some North American species of *Physa* to modify their life history (specifically, by delaying reproduction while growing) in response to crushing predators such as crayfish (CROWL & COVICH 1990). Direct predation by *D. polychroa* on *P. acuta* eggs, while not impossible, was likely negligible (LOMBARDO et al. 2011b). Further declines in *P. acuta* oviposition in our predator-present aquaria may be due to a combination of direct predation and predator-induced decreased activity for larger, reproductive-age snails. Such negative effects of planarias were quantitatively and qualitatively similar to the effect of substratum and/or food limitation occurring once the control *P. acuta* populations reached carrying capacity in wk₄, as treatment-vs.-control differences in weekly oviposition realigned to non-significant levels after wk₂ (Fig. 1). The combined direct predation by *D. polychroa* on hatchling and juvenile snails, and the likely indirect inhibitory effects on larger, reproductive-age snails, eventually led to the local extinction of *P. acuta* by precluding population turnover (Fig. 2), despite a likely negligible direct effect on snail eggs (LOMBARDO et al. 2011b).

Our results support the high predation rates on freshwater snails found in other laboratory investigations (KESLER & MUNNS 1989, TRIPET & PERRIN 1994), but do not support the natural coexistence of

dugesiid planarias and pulmonate snails observed in the field (PYEFINCH 1937, MACAN 1977, MASTRANTUONO & MANCINELLI 2005, authors' personal observations). The interpretation of our results in the light of the available decades-old literature on planarian-snail interactions and our own observations in the field lead to multifaceted considerations and intertwining aspects. General result agreement with findings from earlier laboratory investigations suggests that our own experimental setting, though larger than "typical" Petri dishes (TRIPET & PERRIN 1994), or than the ~2.5 l aquaria in KESLER & MUNNS's (1989) experiment, may have remained unnaturally small, with predator-prey encounters thus more frequent than in the wild. However, our ~4 l aquaria with small-bodied organisms provided a larger arena than KESLER & MUNNS's (1989) ~2.5 l aquaria with much larger-bodied lymnaeid gastropods, and may represent the largest experimental arena (i.e. the closest to natural conditions) for this kind of investigations to date.

As in the studies of KESLER & MUNN (1989), who also added some substratum (lettuce leaves) to provide cover, spatial heterogeneity in our experimental aquaria may have remained too low to protect snails from predation. Our *M. spicatum* sprig appeared to be a refuge habitat, as the vast majority of the few surviving snails in end-of-experiment planarian-present aquaria were found on it, while planarias appeared to avoid the soft-textured sprig. (The latter observation agrees with an apparent natural "preference" for hard substrata by *D. polychroa*: e.g. MASTRANTUONO & MANCINELLI 2005, KIPP 2007, and authors' personal observations.) However, hungry snails may have wandered away from the sprig in search of food, or they may have been weakened and rendered more vulnerable via indirect inhibition of (foraging) activity.

Additionally or alternatively, planarias may have "superspecialised" as active predators on snails following the initial exposure to the experimental no-choice conditions, thus behaving like switch predators (sensu MURDOCH et al. 1975). An apparent overspecialisation of *D. polychroa* on gastropods in snail-rich British habitats (REYNOLDS & YOUNG 1965) but on tubificids (by far the most abundant benthic taxon) in gastropod-poor Toronto Harbour (BODDINGTON & METTRICK 1974) strongly suggests a high degree of trophic opportunism in addition to supporting our hypothesis. LOMBARDO et al. (unpublished) did not find significant predation on live *P. acuta* and other snail species using *D. polychroa* freshly (24 h) collected from multiple-prey culturing aquaria, and TRIPET & PERRIN (1994) observed significant predation by *Dugesia lugubris* long habituated to a *P. acuta*, ad-libitum mono-diet in culturing conditions, also supporting our hypothesis, which however needs to be tested with an ad hoc study. If *D. polychroa* behaved as a switch predator, predatory pressure in-

creased with time in the experimental aquaria, and may have led to unnaturally high predation rates on snails.

However, individual body growth for *D. polychroa* is allometric, with lengthwise growth accompanied by quicker lateral growth, leading to disproportionately wider large planarias, especially when the food supply is abundant (BODDINGTON & METTRICK 1974, authors' personal observations). *D. polychroa* individuals released from intraspecific competition for food (which is probably the main factor regulating free-living freshwater planarias: REYNOLDS & YOUNG 1963, 1965, BODDINGTON & METTRICK 1974, GEE et al. 1998) therefore grow progressively "fatter", implying an ever-increasing demand for maintenance energy. *D. polychroa*'s individual allometric growth supports its possible behaviour as a switch predator and is in agreement with VERBERK et al.'s (2008) view of *D. polychroa* as a high-maintenance taxon. We have observed a marked allometric growth in the experimental *D. polychroa* approximately halfway through the experiment, indeed suggesting high food intake. Peaks in body growth in un-manipulated dugesiid field populations are typically observed in spring, when planarian densities are low and food availability high (REYNOLDS & YOUNG 1963, 1965, BODDINGTON & METTRICK 1974, GEE et al. 1998), suggesting that high planarian predation rates occur naturally at least during parts of the year. *D. polychroa*'s individual allometric growth, its trophic opportunism, fast population growth to reach carrying capacity (REYNOLDS 1977), and high adaptability to the available food supply also suggest that *D. polychroa*, like most other dugesiid planarias, may quickly respond to and take advantage of pulse food inputs. In this light, the high predation pressure by *D. polychroa* on *P. acuta* in our experimental aquaria may easily reflect natural conditions following release from benthivorous fish predation on snails following typical benthic food-web biomanipulation (BRÖNMARK & WEISNER 1992, HOSPER 1997). Our 4 l experimental aquaria thus may have provided a sufficiently large arena to reflect realistic conditions associated with biomanipulation-derived or springtime snail densities. The temporal extent of such a strong regulatory effect of *D. polychroa* on *P. acuta* remains however unknown, with ad hoc, more comprehensive studies needed to formulate a satisfactory answer. An approach with experiments using larger and/or more spatially heterogeneous aquaria may provide an acceptable combination of controlled and realistic conditions to investigate the mechanism(s) regulating dugesiid-gastropod interactions.

Whatever the mechanism(s) involved, *D. polychroa* effectively prevented the small, young-rich, "colonising" populations of *P. acuta* from establishing in the experimental aquaria (Figs 1 and 2), strongly suggesting that *D. polychroa* may preclude the (re)colonis-

ation of *P. acuta* in hard-substratum, spatially homogeneous natural habitats. Absence of *P. acuta* (not even as old, empty shells washed ashore) at the triclad-rich collection site in Lake Piediluco since the early reconnaissance visits in 2007 supports our hypothesis. *P. acuta* is common and widespread in other calcium-rich lakes throughout central Italy, where we have never observed it coexisting with *D. polychroa* (though abundant *P. acuta* may coexist with as abundant populations of the non-native *D. tigrina*, as at the collection site in nearby Lake Ventina). Numerical rarity of otherwise common pulmonate species at the dugesiid-rich collection site in Lake Piediluco also suggests some predatory (or otherwise regulatory) action of *D. polychroa* on other pulmonate snails, while the commonness of the prosobranch *B. leachii* at the site suggests that the operculum may be an effective defence against dugesiid predation. However, other potential snail predators (e.g. leeches) were present at the site, and specific studies are needed to support this hypothesis.

While our results may explain the absence of *P. acuta* in Lake Piediluco and at other similar stony-bottomed, dugesiid-rich sites, *D. polychroa*'s aversion to yielding substrata (e.g. macrophyte sprigs) suggests that this dugesiid may allow the (re)establishment of *P. acuta* in soft-substratum (i.e. habitats con-

ducive to macrophyte (re)growth) and/or vegetation-rich habitats. The occasional presence of *D. polychroa* in such habitats (MASTRANTUONO & MANCINELLI 2005) thus may not influence the (re)establishment of littoral pulmonates — and of the potential keystone *P. acuta* in particular — in recently biomanipulated, soft-bottom shallow lakes, where the recovery of the submerged vegetation is also actively sought for management purposes (BRÖNMARK & WEISNER 1992, LOMBARDO 2005).

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REFERENCES

- ALEXANDER J. E. JR., COVICH A. P. 1991a. Predator avoidance by the freshwater snail *Physella virgata* in response to the crayfish *Procambarus simulans*. *Oecologia* 87: 435–442. doi: 10.1007/BF00634603
- ALEXANDER J. E. JR., COVICH A. P. 1991b. Predation risk and avoidance behavior in two freshwater snails. *Biol. Bull.* 180: 387–393. doi: 10.2307/1542339
- BALL I. R., REYNOLDS T. B. 1981. *British Planarians, Platyhelminthes, Tricladida: keys and notes for the identification of the species.* Cambridge University Press, Cambridge, UK.
- BODDINGTON M. J., METTRICK M. F. 1974. The distribution, abundance, feeding habits, and population biology of the immigrant triclad *Dugesia polychroa* (Platyhelminthes: Turbellaria) in Toronto Harbour, Canada. *J. Anim. Ecol.* 43: 681–699. doi: 10.2307/3531
- BRÖNMARK C., MALMQVIST B. 1986. Interactions between the leech *Glossiphonia complanata* and its gastropod prey. *Oecologia* 69: 268–276. doi: 10.1007/BF00377633
- BRÖNMARK C., WEISNER S. E. B. 1992. Indirect effects of fish community structure on submerged vegetation in shallow, eutrophic lakes: an alternative mechanism. *Hydrobiologia* 243/244: 293–301. doi: 10.1007/BF00007045
- BROWN K. M., LYDEARD C. 2010. Mollusca: Gastropoda. In: THORP J. H., COVICH A. P. (eds). *Ecology and classification of North American freshwater invertebrates.* 3rd ed., Academic Press/Elsevier, Amsterdam, pp. 277–307. doi: 10.1016/B978-0-12-374855-3.00010-8
- CASH K. J., MCKEE M. H., WRONA F. J. 1993. Short- and long-term consequences of grouping and group foraging in the free-living flatworm *Dugesia tigrina*. *J. Anim. Ecol.* 62: 529–535. doi: 10.2307/5202
- CIANFANELLI S., LORI E., BODON M. 2007. Non-indigenous freshwater molluscs and their distribution in Italy. In: GHERARDI F. (ed.). *Biological invaders in inland waters: profiles, distribution and threats.* Springer, Dordrecht, pp. 103–121. doi: 10.1007/978-1-4020-6029-8_5
- COSTIL K., CLEMENT B. 1996. Relationship between freshwater gastropods and plant communities reflecting various trophic levels. *Hydrobiologia* 321: 7–16. doi: 10.1007/BF00018672
- CROWL T. A., COVICH A. P. 1990. Predator-induced life-history shifts in a freshwater snail. *Science* 247: 949–951. doi: 10.1126/science.247.4945.949
- DILLON R. T. JR. 2006. Freshwater gastropoda. In: STURM C. F., PEARCE T. A., VALDÉS A. (eds). *The mollusks: a guide to their study, collection, and preservation.* The American Malacological Society, Pittsburgh, PA, pp. 251–259.
- DILLON R. T. JR., WETHINGTON A. R., RHETT J. M., SMITH T. P. 2002. Populations of the European freshwater pulmonate *Physa acuta* are not reproductively isolated from American *Physa heterostropha* or *Physa integra*. *Invert. Biol.* 121: 226–234. doi: 10.1111/j.1744-7410.2002.tb00062.x



- GAINO E., CIANFICCONI F., CORALLINI SORCETTI C., LANCIONI T., TODINI B., REBORA M., CHIAPPAFREDDO U., BICCHIERAI M.C., SPINELLI G. 2001. Lago di Piediluco: Monitoraggio della Fauna del Canneto (Poriferi e Macroinvertebrati), 1999–2000. Unpublished report, Dept. Anim. Biol. Ecol., University of Perugia, Italy.
- GARCÍA-BERTHOUE E., BOIX D., CLAVERO M. 2007. Non-indigenous animal species naturalized in Iberian inland waters. In: GHERARDI F. (ed.). Biological invaders in inland waters: profiles, distribution and threats. Springer, Dordrecht, pp. 123–140. doi: 10.1007/978-1-4020-6029-8_6
- GEE R. D., PICKAVANCE J. R., YOUNG J. O. 1998. A comparative study of the population biology of the American immigrant triclad *Dugesia tigrina* (Girard) in two British lakes. *Hydrobiologia* 361: 135–143. doi: 10.1023/A:1003170201065
- GEE R. D., YOUNG J. O. 1993. The food niches of the invasive *Dugesia tigrina* (Girard) and indigenous *Polycelis tenuis* Ijima and *P. nigra* (Müller) (Turbellaria; Tricladida) in a Welsh lake. *Hydrobiologia* 254: 99–106. doi: 10.1007/BF00014313
- GIROD A., BIANCHI I., MARIANI M. 1980. Guide per il riconoscimento delle specie animali delle acque interne italiane. 7. Gasteropodi, 1. (Pulmonata; Prosobranchia: Neritidae, Viviparidae, Bithyniidae, Valvatidae). CNR Publication # AQ/1/44; Consiglio Nazionale delle Ricerche, Verona.
- GLÖER P., MEIER-BROOK C. 2003. Süßwassermollusken. Deutscher Jugendbund für Naturbeobachtung, Hamburg.
- HOSPER S. H. 1997. Clearing lakes – an ecosystem approach to the restoration and management of shallow lakes in the Netherlands. Institute for Inland Water Management and Waste Water Treatment (RIZA), Lelystad, NL.
- JENNINGS J. B. 1957. Studies on feeding, digestion, and food storage in free-living flatworms (Platyhelminthes: Turbellaria). *Biol. Bull.* 112: 63–80. doi: 10.2307/1538879
- JONES J. I., YOUNG J. O., HAYNES G. M., MOSS B., EATON J. W., HARDWICK K. J. 1999. Do submerged aquatic plants influence their periphyton to enhance the growth and reproduction of invertebrate mutualists? *Oecologia* 120: 463–474. doi: 10.1007/s004420050879
- KEFFORD B. J., PAPAS P. J., NUGEGODA D. 2003. Relative salinity tolerance of macroinvertebrates from the Barwon River, Victoria, Australia. *Mar. Freshwater Res.* 54: 755–765. doi: 10.1071/MF02081
- KELLY P. M., CORY J. S. 1987. Operculum closing as a defence against predatory leeches in four British freshwater prosobranch snails. *Hydrobiologia* 144: 121–124. doi: 10.1007/BF00014525
- KESLER D. H., MUNNS W. R. JR. 1989. Predation by *Belostomatidae* (Hemiptera): an important cause of mortality in freshwater snails. *J. N. Amer. Benthol. Soc.* 8: 342–350. doi: 10.2307/1467497
- KIPP R. M. 2007. *Dugesia polychroa*. USGS Nonindigenous Aquatic Species Database, Gainesville, FL. Available at <http://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=2359> [last accessed 10 Feb. 2012].
- KOPOWITZ H. 1970. Feeding behaviour and the role of the brain in the polyclad flatworm, *Planocera gilchristi*. *Anim. Behav.* 18: 31–35. doi: 10.1016/0003-3472(70)90066-7
- LOMBARDO P. 1995. Substrate preference of littoral macroinvertebrates during seasonal succession. Unpublished MS Thesis, Kent State University, Kent, OH.
- LOMBARDO P. 2001. Effects of freshwater gastropods on epiphyton, macrophytes, and water transparency under meso- to eutrophic conditions. PhD Dissertation, Kent State University, Kent, OH.
- LOMBARDO P. 2005. Applicability of littoral food-web bio-manipulation for lake management purposes: snails, macrophytes, and water transparency in northeast Ohio shallow lakes. *Lake Reserv. Manage.* 21: 186–202. doi: 10.1080/07438140509354428
- LOMBARDO P., COOKE G. D. 2002. Consumption and preference of selected food types by two freshwater gastropod species. *Arch. Hydrobiol.* 155: 667–685.
- LOMBARDO P., GIUSTINI M., MICCOLI F. P., CICOLANI B. 2011a. Fine-scale differences in diel activity among nocturnal freshwater planarians (Platyhelminthes: Tricladida). *J. Circad. Rhythms* 9: 2. doi: 10.1186/1740-3391-9-2
- LOMBARDO P., MICCOLI F. P., GIUSTINI M., CICOLANI B. 2011b. Planarian (*Dugesia polychroa*) predation on freshwater gastropod eggs depends on prey species, clutch morphology, and egg size. *Fundam. Appl. Limnol.* 178: 325–339. doi: 10.1127/1863-9135/2011/0178-0325
- MACAN T. T. 1977. The influence of predation on the composition of fresh-water animal communities. *Biol. Rev.* 52: 45–70. doi: 10.1111/j.1469-185X.1977.tb01345.x
- MASTRANTUONO L., MANCINELLI T. 2005. Littoral invertebrates associated with aquatic plants and bioassessment of ecological status in Lake Bracciano (Central Italy). *J. Limnol.* 64: 43–53. doi: 10.4081/jlimnol.2005.43
- MCCOLLUM E. W., CROWDER L. B., MCCOLLUM S. A. 1998. Complex interactions of fish, snails, and littoral zone periphyton. *Ecology* 79: 1980–1994. doi: 10.1890/0012-9658(1998)079[1980:CIOFSA]2.0.CO;2
- MICHALIK-KUCHARZ A. 2008. The occurrence and distribution of freshwater snails in a heavily industrialised region of Poland (Upper Silesia). *Limnologica* 38: 43–55. doi: 10.1016/j.limno.2007.09.003
- MOELLER R. E., BURKHOLDER J. M., WETZEL R. G. 1988. Significance of sedimentary phosphorus to a submersed freshwater macrophyte (*Najas flexilis*) and its algal epiphytes. *Aquat. Bot.* 32: 261–281. doi: 10.1016/0304-3770(88)90120-9
- MURDOCH W. W., AVERY S., SMYTH M. E. B. 1975. Switching in predatory fish. *Ecology* 56: 1094–1105. doi: 10.2307/1936149
- NORENA JANSSEN C. 2011. Fauna Europaea: Platyhelminthes: Turbellaria. Fauna Europaea v2.4, www.faunaeur.org [last accessed 10 Feb. 2012].
- PERRIN N. 1986. Les paramètres du cycle vital de *Physa acuta* (Gastropoda, Mollusca) en milieu expérimental. *Rev. Suisse Zool.* 93: 725–736.
- PONGRATZ N., STORHAS M., CARRANZA S., MICHIELS N. K. 2003. Phylogeography of competing sexual and parthenogenetic forms of a freshwater flatworm: patterns and ex-



- planations. *BMC Evol. Biol.* 3: 23. doi: 10.1186/1471-2148-3-23
- PYEFINCH K. A. 1937. The fresh and brackish waters of Bardsey Island (North Wales): a chemical and faunistic survey. *J. Anim. Ecol.* 6: 115–137. doi: 10.2307/1064
- REAVELL P. 1980. A study of the diets of some British freshwater gastropods. *J. Conchol.* 30: 253–271.
- REYNOLDSON T. B. 1977. The population dynamics of *Dugesia polychroa* (Schmidt) (Turbellaria Tricladida) in a recently constructed Anglesey pond. *J. Anim. Ecol.* 46: 63–77. doi: 10.2307/3947
- REYNOLDSON T. B., YOUNG J. O. 1963. The food of four species of lake-dwelling triclads. *J. Anim. Ecol.* 32: 175–191. doi: 10.2307/2533
- REYNOLDSON T. B., YOUNG J. O. 1965. Food supply as a factor regulating population size in freshwater triclads. *Mitt. Int. Verein. Theor. Angew. Limnol.* 13: 3–20.
- SEABY R. M. H., MARTIN A. J., YOUNG J. O. 1995. The reaction time of leech and triclad species to crushed prey and the significance of this for their coexistence in British lakes. *Freshwater Biol.* 34: 21–28. doi: 10.1111/j.1365-2427.1995.tb00419.x
- SCHEFFER M. 1998. Ecology of shallow lakes; reprinted with corrections 2004. Kluwer Academic Publishers, Dordrecht.
- SHELDON S. P. 1987. The effects of herbivorous snails on submerged macrophyte communities in Minnesota lakes. *Ecology* 68: 1920–1931. doi: 10.2307/1939883
- TAYLOR D. W. 2003. Introduction to Physidae (Gastropoda: Hygrophila): biogeography, classification, morphology. *Rev. Biol. Trop.* 51 Suppl. 1: 1–287.
- TRIPET F., PERRIN N. 1994. Size-dependent predation by *Dugesia lugubris* (Turbellaria) on *Physa acuta* (Gastropoda): experiments and model. *Funct. Ecol.* 8: 458–463. doi: 10.2307/2390069
- TURNER A. W., FETTEROLF S. A., BERNOT R. J. 1999. Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. *Oecologia* 118: 242–247. doi: 10.1007/s004420050724
- VERBERK W. C. R. P., SIEPEL H., ESSELINK H. 2008. Life-history strategies in freshwater macroinvertebrates. *Freshwater Biol.* 53: 1722–1738. doi: 10.1111/j.1365-2427.2008.02035.x
- WETHINGTON A. R., LYDEARD C. 2007. A molecular phylogeny of Physidae (Gastropoda: Basommatophora) based on mitochondrial DNA sequences. *J. Moll. Stud.* 73: 241–257. doi: 10.1093/mollus/eym021
- WILKEN G. B., APPLETON C. C. 1991. Avoidance responses of some indigenous and exotic freshwater pulmonate snails to leech predation in South Africa. *S. Afric. J. Zool.* 26: 6–10.
- YOUNG J. O., REYNOLDSON T. B. 1999. Continuing dispersal of freshwater triclads (Platyhelminthes; Turbellaria) in Britain with particular reference to lakes. *Freshwater Biol.* 42: 247–262.
- ZAR J. H. 2009. *Biostatistical Analysis*, 5th ed. Prentice Hall/Pearson, Upper Saddle River, NJ.

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APPENDIX

Table A1. Average (\pm SE) background conditions during the 6-week experiment, by experimental condition (treatment and control: top) and by block (blocks 1 through 4: bottom). T – water temperature; DO – dissolved oxygen concentration; %DO – percent saturation of dissolved oxygen; Macr – macrophyte sprig biovolume. Week-5 pH, DO, and %DO are missing because of temporary instrument failure (NA – not available)

		t_0 (11 Jun)	week 1 (18 Jun)	week 2 (25 Jun)	week 3 (2 Jul)	week 4 (9 Jul)	week 5 (16 Jul)	week 6 (25 Jul)
T (°C)	treat	—	20.1 \pm 0.1	22.4 \pm 0.1	22.0 \pm 0.0	20.6 \pm 0.1	19.2 \pm 0.1	18.2 \pm 0.1
	ctrl	—	20.2 \pm 0.1	22.4 \pm 0.1	22.1 \pm 0.1	20.6 \pm 0.1	19.2 \pm 0.1	18.3 \pm 0.0
pH	treat	—	7.7 \pm 0.0	7.4 \pm 0.1	7.5 \pm 0.1	7.3 \pm 0.1	NA	7.6 \pm 0.1
	ctrl	—	7.7 \pm 0.0	7.5 \pm 0.1	7.6 \pm 0.1	7.3 \pm 0.1	NA	7.5 \pm 0.1
DO (mg l ⁻¹)	treat	—	7.9 \pm 0.1	9.0 \pm 0.1	9.3 \pm 0.1	11.0 \pm 0.2	NA	12.2 \pm 0.2
	ctrl	—	7.8 \pm 0.1	8.9 \pm 0.1	9.1 \pm 0.1	11.0 \pm 0.2	NA	12.8 \pm 0.1
%DO	treat	—	87.3 \pm 0.8	103.7 \pm 1.0	106.8 \pm 1.3	122.1 \pm 1.7	NA	130.2 \pm 2.1
	ctrl	—	86.7 \pm 0.7	102.9 \pm 1.0	104.5 \pm 1.0	122.5 \pm 1.6	NA	135.7 \pm 0.6
Macr (ml)	treat	3.4 \pm 0.1	4.4 \pm 0.1	5.0 \pm 0.1	5.5 \pm 0.1	6.2 \pm 0.2	6.8 \pm 0.3	—
	ctrl	3.3 \pm 0.1	4.5 \pm 0.2	5.0 \pm 0.1	5.4 \pm 0.1	6.4 \pm 0.2	7.4 \pm 0.3	—

		t_0 (11 Jun)	week 1 (18 Jun)	week 2 (25 Jun)	week 3 (2 Jul)	week 4 (9 Jul)	week 5 (16 Jul)	week 6 (25 Jul)
T (°C)	1	—	20.2 \pm 0.1	22.1 \pm 0.0	21.9 \pm 0.0	20.3 \pm 0.0	18.7 \pm 0.0	18.2 \pm 0.0
	2	—	20.0 \pm 0.0	22.3 \pm 0.0	22.0 \pm 0.1	20.5 \pm 0.0	19.1 \pm 0.1	18.3 \pm 0.0
	3	—	20.1 \pm 0.1	22.5 \pm 0.0	22.1 \pm 0.0	20.6 \pm 0.0	19.3 \pm 0.0	18.4 \pm 0.0
	4	—	20.3 \pm 0.1	22.6 \pm 0.0	22.3 \pm 0.0	20.9 \pm 0.1	19.5 \pm 0.1	18.5 \pm 0.1
pH	1	—	7.8 \pm 0.1	7.8 \pm 0.1	8.0 \pm 0.1	6.9 \pm 0.2	NA	8.0 \pm 0.1
	2	—	7.6 \pm 0.0	7.3 \pm 0.0	7.5 \pm 0.0	7.1 \pm 0.1	NA	7.6 \pm 0.1
	3	—	7.7 \pm 0.0	7.3 \pm 0.0	7.4 \pm 0.0	7.5 \pm 0.0	NA	7.2 \pm 0.0
	4	—	7.8 \pm 0.0	7.4 \pm 0.0	7.4 \pm 0.0	7.7 \pm 0.0	NA	7.1 \pm 0.0
DO (mg l ⁻¹)	1	—	8.1 \pm 0.1	9.3 \pm 0.0	9.5 \pm 0.2	11.6 \pm 0.1	NA	12.9 \pm 0.1
	2	—	8.1 \pm 0.0	9.0 \pm 0.0	9.2 \pm 0.1	11.0 \pm 0.2	NA	12.4 \pm 0.1
	3	—	7.6 \pm 0.0	8.6 \pm 0.1	8.9 \pm 0.1	10.9 \pm 0.2	NA	13.0 \pm 0.1
	4	—	7.7 \pm 0.0	8.9 \pm 0.1	9.2 \pm 0.0	10.5 \pm 0.1	NA	12.6 \pm 0.0
%DO	1	—	89.7 \pm 0.6	106.3 \pm 0.4	108.2 \pm 2.5	128.4 \pm 1.2	NA	136.8 \pm 0.7
	2	—	89.0 \pm 0.3	104.7 \pm 1.2	105.9 \pm 1.2	121.9 \pm 2.0	NA	132.1 \pm 1.1
	3	—	84.7 \pm 0.4	99.2 \pm 0.9	102.2 \pm 0.6	121.7 \pm 2.0	NA	138.0 \pm 0.5
	4	—	84.6 \pm 0.3	103.2 \pm 0.8	106.4 \pm 0.6	117.3 \pm 1.4	NA	134.6 \pm 0.4
Macr (ml)	1	3.1 \pm 0.1	3.9 \pm 0.1	5.0 \pm 0.0	5.3 \pm 0.1	5.7 \pm 0.2	6.5 \pm 0.3	—
	2	3.4 \pm 0.2	4.2 \pm 0.2	5.3 \pm 0.1	5.2 \pm 0.1	6.3 \pm 0.2	7.7 \pm 0.6	—
	3	3.3 \pm 0.2	4.8 \pm 0.1	4.8 \pm 0.2	5.6 \pm 0.2	6.5 \pm 0.3	7.3 \pm 0.1	—
	4	3.5 \pm 0.2	4.8 \pm 0.2	4.9 \pm 0.2	5.7 \pm 0.2	6.7 \pm 0.3	7.4 \pm 0.6	—

Table A2. Repeated-measures-ANOVAs for background conditions, by block. T – water temperature; DO – dissolved oxygen concentration; %DO – percent saturation of dissolved oxygen; Macr – macrophyte sprig biovolume. Tests were performed on log-transformed data. For all ANOVAs, $df_{\text{block}}=3$, $df_{\text{time}}=5$ ($df_{\text{time}}=4$ for pH, DO and %DO missing wk₅ data), $df_{\text{block} \times \text{time interaction}}=15$ ($df_{\text{interaction}}=12$ for pH, DO and %DO missing wk₅ data), between-subject $df_{\text{error}}=10$, and within-subject $df_{\text{error}}=50$ (within-subject $df_{\text{error}}=40$ for pH, DO and %DO missing wk₅ data)

		T	pH	DO	%DO	Macr
block	<i>F</i>	9.512	5.033	6.846	7.149	10.435
	<i>p</i>	0.003	0.022	0.009	0.008	0.002
time	<i>F</i>	8000.498	22.397	702.069	620.734	92.395
	<i>p</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
block × time interaction	<i>F</i>	14.780	17.400	2.985	3.014	1.417
	<i>p</i>	<0.0001	<0.0001	0.003	0.003	0.169

Table A3. Repeated-measures-ANOVAs for background conditions, by experimental condition. T – water temperature; DO – dissolved oxygen concentration; %DO – percent saturation of dissolved oxygen; Macr – macrophyte sprig biovolume. Tests were performed on log-transformed data. For all ANOVAs, $df_{\text{experimental condition}}=1$, $df_{\text{time}}=5$ ($df_{\text{time}}=4$ for pH, DO and %DO missing wk₅ data), $df_{\text{condition} \times \text{time interaction}}=5$ ($df_{\text{interaction}}=4$ for pH, DO and %DO missing wk₅ data), between-subject $df_{\text{error}}=12$, and within-subject $df_{\text{error}}=60$ (within-subject $df_{\text{error}}=48$ for pH, DO and %DO missing wk₅ data)

		T	pH	DO	%DO	Macr
experimental condition	<i>F</i>	0.794	0.706	0.797	0.768	0.144
	<i>p</i>	0.391	0.417	0.390	0.398	0.711
time	<i>F</i>	933.339	3.831	231.322	205.001	41.869
	<i>p</i>	<0.0001	0.0008	<0.0001	<0.0001	<0.0001
condition × time interaction	<i>F</i>	0.188	0.767	2.164	2.124	1.724
	<i>p</i>	0.966	0.551	0.084	0.089	0.140

Table A4. *P* values for the SNK-based treatment-vs.-control comparisons [equivalent to a one-way ANOVA computed against the model $y=\text{average}(y)$ with $df=1,22$] for each weekly sampling within the repeated-measures-ANOVAs of Table A3, for water temperature (T), pH, dissolved oxygen concentration (DO), oxygen saturation (%DO), and macrophyte sprig biovolume (Macr); NA – not available (temporary instrument failure), and NC – not calculable ($n_{\text{treatment}}$ at wk₆ < 3). Significant treatment-vs.-control for $p \leq 0.05$

	T	pH	DO	%DO	Macr
week 1	0.270	0.244	0.061	0.145	0.140
week 2	0.637	0.647	0.290	0.233	0.974
week 3	0.268	0.624	0.198	0.160	0.963
week 4	0.714	0.196	0.819	0.845	0.957
week 5	0.569	NA	NA	NA	0.456
week 6	0.252	0.703	NC	NC	—