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Detachment, displacement and reattachment activity in a freshwater byssate mussel (*Limnoperna fortunei*): the effects of light, temperature and substratum orientation

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The ability of the freshwater bivalve *Limnoperna fortunei* to voluntarily detach from the substratum, crawl and reattach as a function of illumination, temperature, substratum orientation, and mussel size was investigated. Thirty-two per cent of the 879 experimental animals detached and reattached elsewhere at least once during five- to eight-day experiments. The proportions of mobile mussels were significantly higher in permanent darkness than under permanent illumination. Displacement distances were also higher in darkness, but statistical differences with illuminated individuals were inconclusive. No evidence of circadian rhythms was detected. Mobile mussels were often significantly smaller than non-mobile individuals. It was not possible to detect the effect of water temperature (22°C and 31°C), or substratum orientation (topside and underside) on mussel mobility, but because the power of the statistical tests was low, future experiments are needed to confirm this result. The ability of mussels to voluntarily detach and reattach elsewhere has important implications for biofouling control.

Keywords: *Limnoperna fortunei*; golden mussel; attachment-detachment; byssus; mobility; macrofouling

Introduction

Limnoperna fortunei (Dunker 1857) (the golden mussel) is an invasive freshwater bivalve which was introduced in the Río de la Plata estuary (South America) from Southeast Asia around 1990 (Pastorino et al. 1993). Its dispersion upstream in the Uruguay river, and especially the Paraná-Paraguay rivers, was very fast due to the ability of the adults to attach to the hulls of commercial ships operating along these waterways ('jump dispersal', *cf*. MacIsaac et al. 2001) and subsequently colonizing the basin by means of its downstream drifting planktonic larvae (Boltovskoy et al. 2006). Currently, the golden mussel is present in practically all the Río de la Plata watershed, as well as in several smaller Argentine (Mar Chiquita) and Brazilian–Uruguayan basins (Patos-Mirim, Guaíba, Tramandaí) (Oliveira et al. 2015).

Due to its widespread distribution and high population densities, *L. fortunei* has been associated with significant impacts on the ecosystems invaded, including increased water clarity due to grazing of particulate organic matter, enhancement of macrophyte and periphyton growth, modification of nutrient concentrations and ratios, decrease in phytoplankton production but enhancement of cyanobacterial blooms, increase in sedimentation rates and organic matter content in the sediments, enhancement of the abundance and diversity of benthic invertebrates, and supply of food for larval and adult fish (Boltovskoy & Correa 2015). Furthermore, the rapid spread of *L. fortunei* has brought about significant impacts on most industrial and power plants that use raw water from invaded water bodies. Golden mussels develop massive beds in water conduits, sieves, heat exchangers, filters, trash racks, and other components, clogging them and causing pressure loss, overheating and corrosion (Boltovskoy et al. 2015).

Due to disturbances such as wave impact and predation, byssate bivalves may detach, displace and resettle elsewhere (Dayton 1971; Paine & Levin 1981), as also shown by artificially detached animals, which crawl and reattach (Uryu et al. 1996; Toomey et al. 2002). For *L. fortunei* and other sessile bivalves, voluntary (rather than forcible) detachment has also been observed in experimental settings, presumably as a response to stressful conditions (Mori 1948; Mackie et al. 1989; Cawein 1993; Eckroat et al. 1993; Iwasaki 2015), but the mechanisms that govern the voluntary detachment and translocation of fouling byssate mussels, including the distance traveled and the frequency of these displacements, are poorly known (Eckroat et al. 1993). Most of the data are based on the observation of isolated organisms artificially detached from the substratum (Uryu et al. 1996; Toomey et al. 2002), providing limited insight into the translocation of mussels in natural conditions. Quantification of such behavior is important for a

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better understanding of mussel population dynamics, and particularly for applied studies of antifouling strategies, since fouling by byssate mussels is a major concern for many industrial plants that use raw seawater, river water, or lake water for cooling purposes (Mackie & Claudi 2010; Rajagopal et al. 2012).

The present study examines the ability of *L. fortunei* to detach from the substratum, crawl and reattach elsewhere as a function of (1) illumination, (2) temperature, (3) substratum orientation, and (4) mussel size.

Materials and methods

For all experiments, mussels were collected along the shores of the uppermost freshwater Río de la Plata estuary (34°32.9′S, 58°25.6′W) and immediately transported to the laboratory. Upon arrival, animals were isolated by cutting their byssal threads, taking care to avoid injuring the byssus gland or other internal organs. In order to allow identification of individuals during the experiments, animals were color-coded with permanent non-toxic paint on both valves. Isolated, marked mussels 6.2–24.2 mm in length (Table 1) (>90% 8–19 mm; mussel sizes did not differ significantly between replicates or between experiments, ANOVA $p>0.05$) were distributed evenly and allowed to reattach on PVC panels (15×10 cm) in acclimation aquaria for seven days. Before starting the experiments, panels were examined and dead, unattached and loosely attached animals were removed. Mussel-colonized panels were placed vertically, except in those aimed at assessing the effects of substratum orientation on mobility (see below) in aerated 3.5 l glass aquaria. Animals were not fed during the experiments.

Although the numbers of individuals in each replicate of each experiment were initially identical or very similar, throughout the experiments the proportions of mussels that detached and fell off the plates (and were therefore excluded from the analyses) varied between experiments. Thus, the total numbers of mussels that remained on the plates from start to the end of each experiment (and whose behavior was compared as a function of the different experimental settings: illumination, temperature, orientation) were often different (Table 2). This circumstance was of concern because it raised the possibility that observed movements (or lack thereof) could respond not only to differences in the conditions tested, but also to density-dependent reactions. In order to investigate this issue, additional tests were performed using observations based on three days only (rather than seven to eight days), at which time the numbers of experimental mussels were more similar to the initial target densities, and differed little between replicates (Table 2).

The ability of *L. fortunei* to detach from the substratum, crawl and reattach elsewhere in response to various external stimuli: illumination, temperature, and substratum orientation, was assessed (Figure 1).

Illumination

The influence of light on mussel movements was assessed using two light conditions: permanent illumination (~500 lx) and permanent darkness (Figure 1A and B). For these experiments two replicates with 27 and 47 mussels (light treatment; the third replicate had to be discarded due to high mussel mortality) were used, and three replicates with 52–66 mussels (dark treatment) (in all cases, the numbers of experimental animals refer to those that remained on the plates from the start to the end of each experiment, unless otherwise noted; see 'Effects of mussel densities' below and Table 2). The bivalves used were collected at a water temperature of 17°C and acclimated to experimental temperatures at a rate of 1°C per day. Experiments were conducted in controlled temperature chambers at 23°C and the results analyzed on the basis of photographs taken every 24 h during the first four days, and every 48 h subsequently until day 8.

In order to assess mussel movements at a higher time resolution, additional experiments were performed under permanent illumination, in permanent darkness, and exposed to a normal day–night cycle, but photographs were taken at hourly intervals (Figure 1C–E). These experiments lacked replicates, and therefore the results were not assessed statistically and should be considered as preliminary. Mussels were collected at water temperatures of 22–23°C and monitored at 22°C. Prior to experimentation, all mussels were acclimated for seven days in tanks placed in front of a window facing northeastwards. In the dark treatments, the aquarium and the photo camera were covered by a light-proof box, thus blocking all external light sources (with the exception of the camera flash). In the light treatments, experimental containers were permanently illuminated by a lamp delivering ~500 lx (at the surface of the PVC panel). The day–night cycle experiment was conducted in a glass tank placed in the vicinity of a window facing northeast (but away from direct exposure to sunlight in order to avoid excessive temperature changes, which varied from 21–24°C) and exposed to a natural day–night light cycle (14–10 h; ~0 to 4000 lx, depending on the time of day) (Figure 1E). In these preliminary experiments (Figure 1C–E) mussel detachment and translocation was assessed on the basis of photographs taken at hourly intervals over five days. A small proportion (7%) of the animals that were out of focus or otherwise unrecognizable in the photographs were excluded from the analyses.

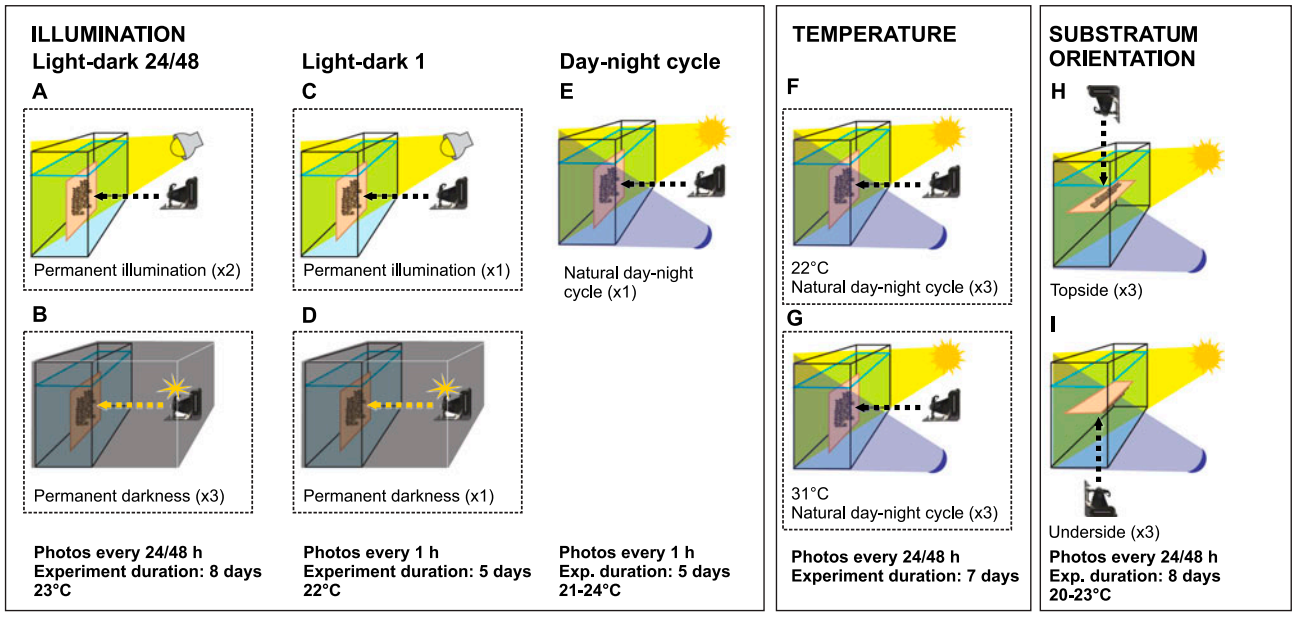

Figure 1. General scheme of the experiments performed. Dashed frames denote experiments in a controlled temperature chamber.

Temperature

The effects of temperature were assessed in seven-day experiments at 22°C and 31°C (roughly typical spring/autumn and summer water temperatures, respectively, in many South American water bodies invaded by *L. fortunei*) (Figure 1F and G). Mussels were collected at a water temperature of 15–17°C, and acclimated at a rate of 1°C per day until reaching experimental temperatures. Three replicates at each temperature were used, comprising 24–63 animals per replicate. All experimental tanks were kept in a controlled temperature chamber with glass doors and exposed to the normal day–night light cycle of a laboratory with large windows (between ~0 and 120 lx, depending on the time of day). Photographs were taken every 24 h during the first three days, and every 48 h thereafter until day 7.

Substratum orientation

The effects of substratum orientation were tested using the same PVC panels as described above, but placed in the tank with the colonized surface facing upwards or downwards (Figure 1H and I). Mussels for these experiments were collected at a temperature of 15–17°C and acclimated to the experimental temperature (20–23°C) as in the previous test. Both acclimation and experimentation were carried out under a natural day–night light cycle (~0 to 100 lx). Panels were located in the center of the tank, away from its walls and the bottom. Three replicates for each orientation with 33–54 mussels per replicate were used. Photographs were taken every 24 h during the first four days, and every 48 h thereafter until day 8. Mussels that moved to the opposite side of the panel were discarded.

Photographs were taken with an automated Canon camera Power Shot G9 (Canon Inc., Tokyo, Japan). Mussel displacements between shots were assessed with the aid of the Tracker (Open Source Physics Project, http://www.opensourcephysics.org/) computer program, which allows estimation of the minimum (Euclidean) distances covered by a moving object between consecutive shots (actual distances may have been somewhat higher due to nonlinear displacement trajectories). In many cases, slight differences in position between shots (up to 16 mm) were found to be due to the animals turning or pivoting around their byssus, rather than detaching from the substratum and reattaching nearby. Thus, a minimum distance of 16 mm was used as the threshold value for all translocation movements.

Since golden mussels do not form multi-layered beds and adult individuals attach to the substratum rather than to the sides of nearby congeners (Correa et al. 2015), it is contended that the translocations observed are effectively the result of individual voluntary detachment and reattachment elsewhere, rather than displacements due to mussels being carried away by their neighbors (Commito et al. 2014).

Mussel translocation activity was assessed using two complementary indicators: (1) the proportion of experimental animals that moved, and (2) the distance covered by the moving mussels. Animals that fell off the plate during the experiment were excluded from all results and not computed in the totals indicated in Table 2 (but see 'Effects of mussel densities' below).

Statistical analyses

Interpretations of the results of experiments with records at 24/48 h intervals were performed with the aid of repeated measures ANOVA (RMA) tests (SPSS program using a significance level of 0.05). When necessary, data were transformed with the *Arcsin* $\sqrt{x}$ (percentage data) or the $Ln\,(x+1)$ (displacement distance data) functions to comply with test assumptions (Table 2).

Estimates based on the effect-sizes of the treatments (very low, with the exception of permanent illumination *vs* permanent darkness, see Table 2), indicated that in order to attain a reasonably high power ($\sim \geq 0.8$), the analyses needed up to 122 replicates, which was obviously prohibitive. Thus, in order to validate the results of the RMA tests, especially in those cases where the significance was marginal, differences between settings were also assessed using an alternative approach, *viz.* generalized linear models (GLM, InfoStat program, http://www.infostat.com.ar). These models are linear in the parameters and random variables, but do not necessarily involve a linear relationship between the response and the explanatory variable (Crawley 1993). GLMs work on the assumption that, for any given dataset, there are several valid models that can explain the data, one of which satisfies the Akaike information criterion (AIC) best (lower AIC values indicate better model fit to the data and lower model complexity) (McCullagh & Nelder 1983). Thus, for each one of the analyses shown in Table 2, approximately seven models were run, choosing the one with the lowest AIC. GLMs have some advantages over RMA, especially when working with proportions, with data with unequal variances, and adapt better to non-Gaussian distributions and unbalanced designs (McCullagh & Nelder 1983). In almost all cases, the results of these new analyses confirmed those obtained with RMA (Table 2).

Experiments with hourly records (Figure 1C–E) were not replicated (each of the latter involved assessing around 8,000 translocations), and therefore no confidence limits are reported for these. However, because their results were in very close agreement with those of the replicated trials, and, furthermore, they suggest additional cause–effect relationships, it was considered of interest to include these preliminary results in the present report.

Results

Effects of mussel densities

In order to investigate the potential effects of variable mussel densities on their mobility under different conditions, comparisons based on 7–8 d observations were repeated using the first 3 d of each experiment only, when mussel numbers were close to their initial target values. Thus, while by the end of the experiments (7–8 d) the mean difference in animals subjected to paired treatments was 38%, on day 3 this difference was only 14% (Table 2). With only one exception, both RMA and GLM showed similar results using 3 and 7–8 d observations, suggesting that dissimilar densities had not affected the results. The exception is mussel displacement distance in light *vs* darkness, which was non-significant after 3 d (RMA and GML), but yielded a significant RMA result after observations for 8 d (Table 2).

Illumination

In permanent darkness (Figure 1B), the proportion of animals that moved (40.1%) was significantly higher than that in the permanently illuminated tanks (16.7%) (Figures 2 and 3; Tables 1 and 2). The overall distance covered by moving mussels was also higher in the dark (mean: 13.1 mm mussel^{-1} d^{-1}), than when illuminated (6.1 mm mussel^{-1} d^{-1}) (Figure 3); this difference was significant according to the RMA analysis ($p = 0.015$), but the GLM model suggested that illumination *vs* darkness had no effect on the distance covered ($p = 0.087$). Neither RMA nor GLM yielded significant differences when using the first three days of these experiments only (Table 2).

The outcome of the complementary test recording the position of the animals at 1 h intervals (Figure 1C and D) confirmed the above results, showing that under permanent darkness, over the five-day experiment 41.4% of the mussels detached and reattached elsewhere (Figure 4, right panel), as opposed to 15.7% in permanent light (Figure 4, left panel; Table 1). The overall distances covered by the end of the experiment were also substantially higher in the dark (28.6 mm mussel^{-1} day^{-1}) than with illumination (18.3 mm mussel^{-1} day^{-1}).

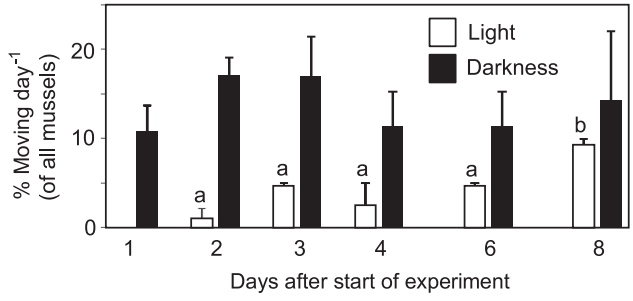

Figure 2. Proportions (means ± SE) of experimental mussels that moved and reattached elsewhere on each of six successive observations under illumination and in the dark (Figure 1A, B). Different letters denote significant differences ($p < 0.05$, GLM and Fisher's LSD contrasts).

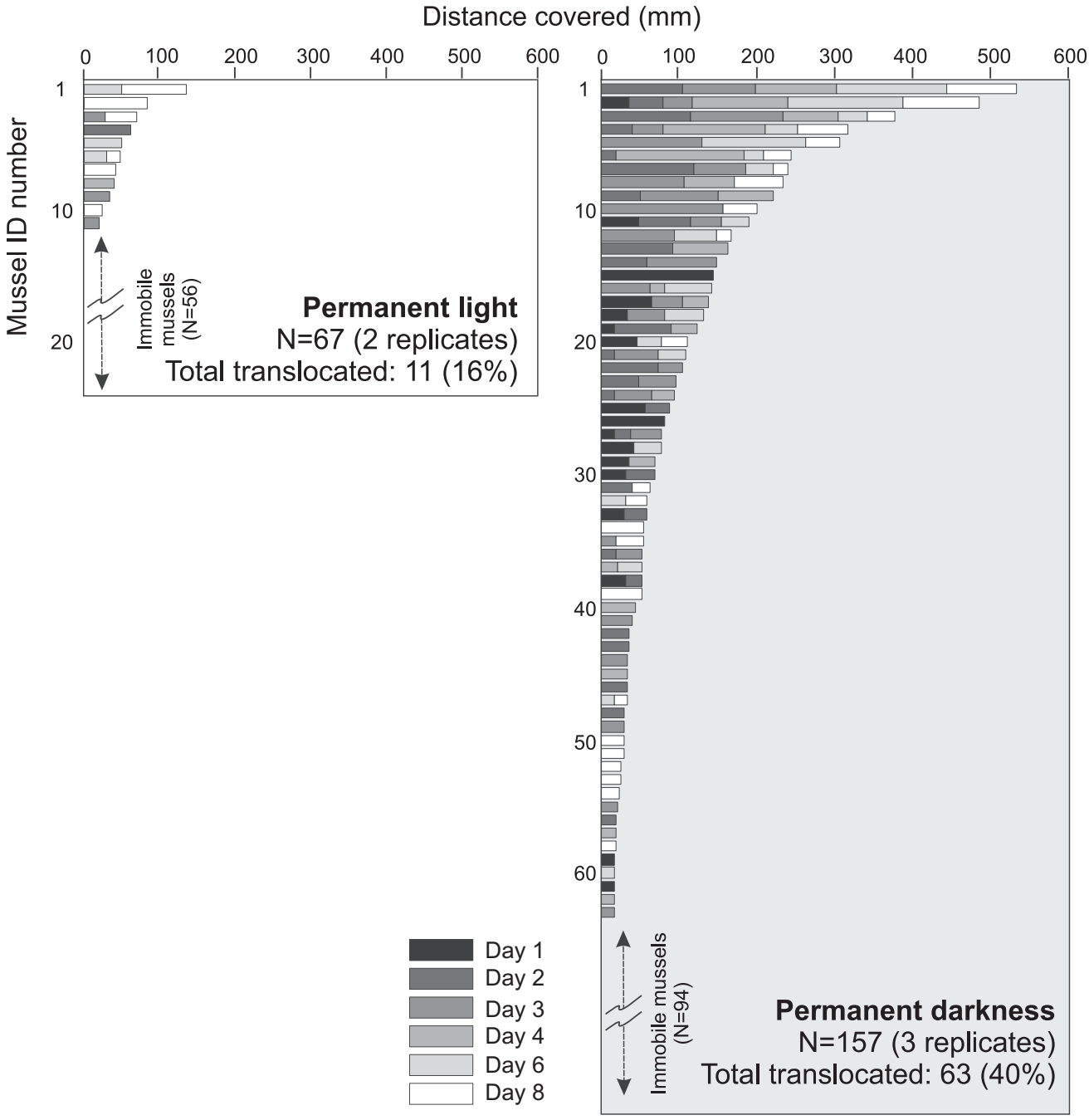

Figure 3. Distances covered by all moving mussels in all replicates under permanent darkness (right panel) and permanently illuminated conditions (left panel) throughout a six-day experiment (observations every 24/48 h; Figure 1A and B). Each bar represents one animal. Successive days are denoted with different shadings.

The effects of light on the behavior of the animals suggested by these results was confirmed by exposures to a normal day–night cycle (Figure 1E). Much higher proportions of the experimental mussels moved during the night (44.0%), than during the day (7.6%) (Figure 5). The distances covered were also slightly higher during the night (39 mm mussel^{-1} day^{-1}) than during the day (30 mm mussel^{-1} day^{-1}).

In order to explore whether mussel mobility was associated with circadian rhythms or was only a response to current lighting conditions, data obtained at 1 h intervals in permanently illuminated and permanently dark settings (Figure 1C and D) were analyzed considering sunrise to sunset, and sunset to sunrise time offsets separately. The corresponding results are illustrated in Figure 4 and Table 1, showing that actual day/night

Table 1. General overview of the results of mussel mobility under different experimental settings.

	Light–dark treatment, permanent light	**Light–dark treatment, permanent darkness**	**Light–dark treatment, permanent light**	**Light–dark treatment, permanent darkness**	**Day–night cycle**	**Temp., 22°C**	**Temp., 31°C**	**Substratum orientation, topside**	**Substratum orientation, underside**
Experimental setting in Figure 1	1A	1B	1C	1D	1E	1F	1G	1H	1I
Experiment duration (h [d])	192 [8.0]	192 [8.0]	119 [5.0]	117 [4.9]	113 [4.7]	168 [7.0]	168 [7.0]	192 [8.0]	192 [8.0]
Interval between records (h)	24/48	24/48	1	1	1	24/48	24/48	24/48	24/48
Percentage of mobile mussels (all replicates) [N]	16.7 [11]	40.1 [63]	15.7 [11]	41.4 [29]	45.5 [30]	52.9 [36]	27.2 [41]	21.8 [22]	23.1 [30]
Distance covered by all mobile mussels by the end of the experiment (mm per mussel per day, all replicates)	6.7	13.1	16.5	22.7	23.7	5.7	12.2	7.4	7.3
Percentage of mussels that moved during daytime hours [N]	—	—	5.7 [4]	21,4 [15]	7.6 [5]	—	—	—	—
Percentage of mussels that moved during nighttime hours [N]	—	—	11.4 [8]	27.1 [19]	44.0 [29]	—	—	—	—
Percentage of mussels that moved during daytime hours only [N]	—	—	4.3 [3]	11.4 [8]	1.5 [1]	—	—	—	—
Percentage of mussels that moved during nighttime hours only [N]	—	—	10 [7]	17.1 [12]	37.9 [25]	—	—	—	—
Percentage of mussels that moved during daytime and nighttime hours [N]	—	—	1.4 [1]	10.0 [7]	6.1 [4]	—	—	—	—
Mean displacement distance during daytime hours (mm; excluding static records)	—	—	29.9	49.2	30	—	—	—	—
Mean displacement distance during nighttime hours (mm; excluding static records)	—	—	39.8	49.3	39	—	—	—	—
Mean length of mobile mussels mm (all replicates)	15	12.2	13.3	12.4	12.8	12.5	12.3	13.1	12.6
Mean length of immobile mussels, mm (all replicates)	14.1	14.3	13.7	15.2	13.6	14	13.4	14.4	13.1
p-value for *t*-test between lengths of mobile and immobile mussels (2-tailed)	0.569	<0.000	0.783	0.001	0.169	0.042	0.020	0.120	0.100
Total movements recorded (pooled data for all replicates)	14	130	26	65	77	49	63	34	49
% movements upwards	62.5	66.9	56.0	50.8	66.2	36.7	64.9	—	—
% movements downwards	37.5	33.1	40.0	49.2	33.8	63.3	35.1	—	—

Table 2. Statistical values (repeated measures ANOVA, RMA, and generalized linear models, GLM) for the effects of physical factors on the proportions of mobile mussels and displacement distances.

Treatment	Experiment duration (d)	Replicates	N per replicate	Mean N per treatment	Data transformation (RMA and GLM)	RMA F	RMA P	GLM p	Effect-size	Figure
Effects of light, temperature and orientation										
Perm. light *vs* perm. dark, % mobile animals	8	2–3	46, 20 - 51, 52, 54	33–52	Arcsin (√x)	15.239	0.030	0.038	0.894	2, 3
Perm. light *vs* perm. dark, displ. distance*	8	2–3	46, 20 - 51, 52, 54	33–52	Ln (x+1)	25.348	0.015	0.087	0.836	2, 3
Temperature, 22 *vs* 31°C, % mobile animals	7	3	26, 13, 29 - 55, 53, 43	23–50	Arcsin (√x)	1.493	0.289	0.307	0.272	6
Temperature, 22 *vs* 31°C, displ. distance	7	3	26, 13, 29 - 55, 53, 43	23–50	No transf.	7.427	0.053	0.953	0.650	6
Topside *vs* underside, % mobile animals	8	3	30, 34, 37 - 51, 32, 47	34–43	Arcsin (√x)	1.535	0.283	0.304	0.277	7
Topside *vs* underside, displ. distance	8	3	30, 34, 37 - 51, 32, 47	34–43	No transf.	3.445	0.137	0.144	0.463	7
Perm. light *vs* perm. dark, % mobile animals	3	2–3	64, 44 - 55, 59, 63	54–59	Arcsin (√x)	42.515	0.007	0.013	0.934	
Perm. light *vs* perm. dark, displ. distance *	3	2–3	64, 44 - 55, 59, 64	54–59	Ln (x+1)	2.575	0.207	0.257	0.462	
Temperature, 22 *vs* 31°C, % mobile animals	3	3	47, 34, 43 - 57, 58, 46	41–54	Arcsin (√x)	3.668	0.128	0.204	0.478	
Temperature, 22 *vs* 31°C, displ. distance	3	3	47, 34, 43 - 57, 58, 46	41–54	No transf.	0.064	0.813	0.813	0.015	
Topside *vs* underside, % mobile animals	3	3	40, 50, 60 - 60, 49, 57	50–55	Arcsin (√x)	0.078	0.794	0.794	0.019	
Topside *vs* underside, displ. distance	3	3	40, 50, 60 - 60, 49, 57	50–55	No transf.	0.245	0.647	0.567	0.058	
Differences between consecutive days, % mobile animals										
Perm. light (24/48 h intervals)	8	2	46, 20	33	Arcsin (√x)	3.633	0.308	0.005	0.797	1A, 2
Perm. dark (24/48 h intervals)	8	3	51, 52, 54	52	Arcsin (√x)	0.609	0.557	0.499	0.233	1B, 2
Temperature, 22°C	7	3	26, 13, 29	23	Arcsin (√x)	1.707	0.297	0.238	0.460	1F, 6
Temperature, 31°C	7	3	55, 53, 43	50	Arcsin (√x)	1.913	0.295	0.202	0.489	1G, 6
Topside	8	3	30, 34, 37	34	Arcsin (√x)	2.231	0.237	0.131	0.527	1H, 7
Underside	8	3	51, 32, 47	43	Arcsin (√x)	2.926	0.197	<0,0001	0.594	1I, 7
Differences between consecutive days, displacement distance										
Perm. light (24/48 h intervals)	8	2	46, 20	33	Ln (x+1)	1.256	0.464	0.389	0.557	1A, 2
Perm. dark (24/48 h intervals)	8	3	51, 52, 54	52	Ln (x+1)	2.050	0.269	0.156	0.506	1B, 2
Temperature, 22°C	7	3	26, 13, 29	23	No transf.	0.387	0.670	0.159	0.162	1F, 6
Temperature, 31°C	7	3	55, 53, 43	50	No transf.	1.389	0.352	0.834	0.410	1G, 6
Topside	8	3	30, 34, 37	34	No transf.	1.896	0.269	0.182	0.487	1H, 7
Underside	8	3	51, 32, 47	43	No transf.	0.509	0.560	0.642	0.203	1I, 7

Notes: Results of analyses of the effects of the variables investigated are shown for the entire span of each experiment (seven or eight days, excluding mussels which fell off the plates before experiment termination, and whose N values are therefore more dissimilar between treatments; first six rows), and for the first three days of each experiment only (where the numbers of animals excluded from the analyses are much lower and therefore the N values differ little between treatments, rows 7–12). Experiments without replicates are not included. *Based on mobile mussels only.

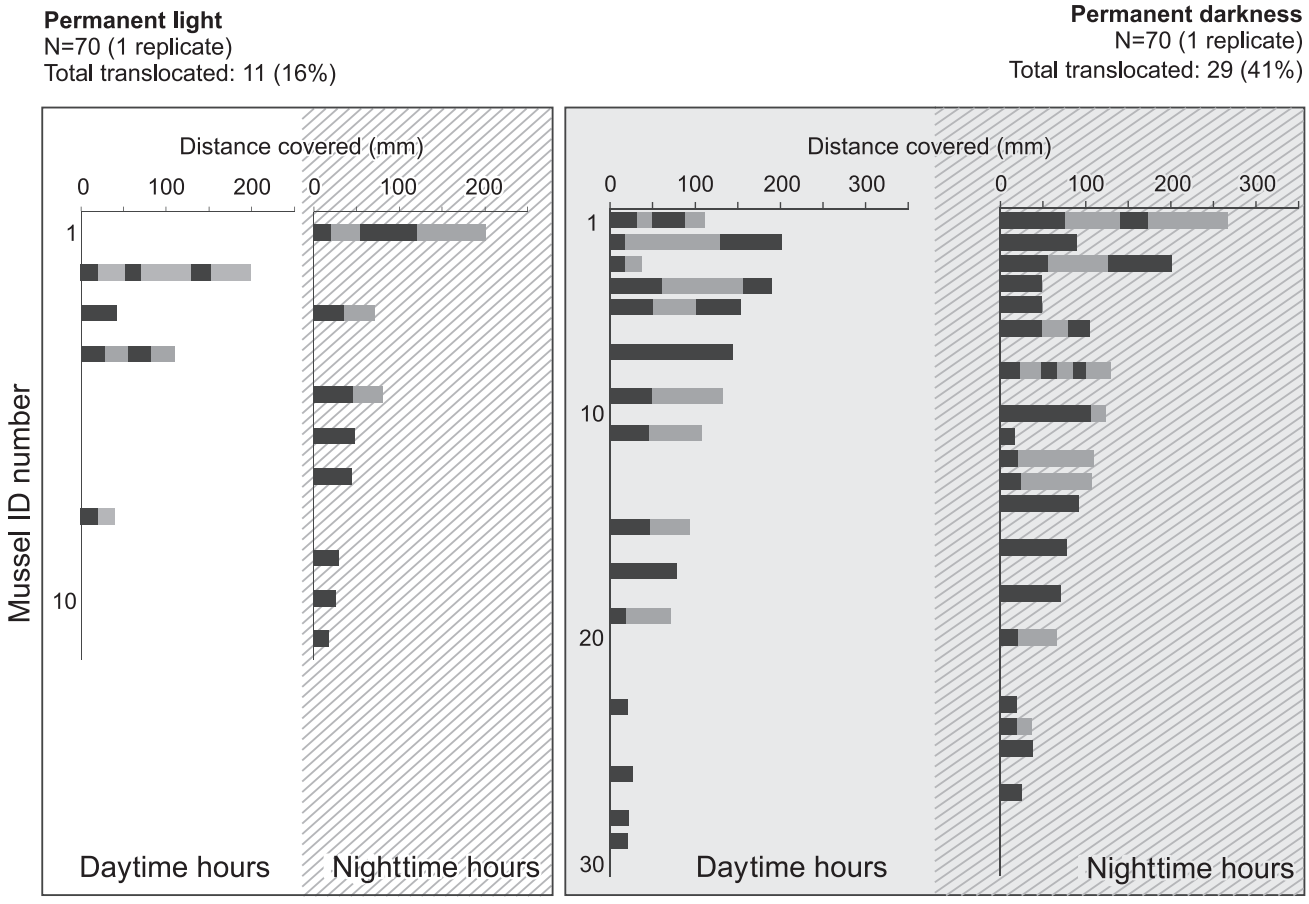

Figure 4. Distances covered by all moving mussels under permanent darkness (right panel) and permanently illuminated conditions (left panel) throughout a five-day experiment (observations every 1 h; Figure 1C, D). In both cases, displacements occurring during daytime (sunrise to sunset, non-hatched) and during nighttime hours (sunset to sunrise, hatched) are indicated separately. Each bar represents one animal. Different shadings denote discrete mussel translocations (not necessarily consecutive). Immobility periods are omitted.

periods seemed to have no effect on mussel mobility. This suggests that the differences observed in the five-day experiment under a natural day–night cycle (Figures 1E and 5) were solely due to changes in illumination.

Temperature

Throughout the seven-day experiment (Figure 1F and G), the proportions of translocating mussels did not differ significantly at 22°C (52.9%) or at 31°C (27.1%) (Figure 6; Tables 1 and 2). The overall distances covered by moving animals were higher at 31°C (12.2 mm mussel^{-1} day^{-1}) than at 22°C (5.7 mm mussel^{-1} day^{-1}), but this difference was not significant either (Figure 6; Table 2).

Substratum orientation

The proportions of mobile mussels on the topside and the underside of the plates (Figure 1H and I) were very similar (21.8 and 23.1%, respectively), as were the distances covered (7.4 and 7.3 mm mussel^{-1} day^{-1}, respectively) (Figure 7; Tables 1 and 2).

Mussel size

Four (of nine) paired comparisons showed that mobile mussels differed significantly in size from the immobile individuals. In all these cases, as well as in four out of the five where differences in size were not statistically significant, mobile animals were smaller than those that never moved (Figure 8; Table 1).

Direction of movement

In six (of seven) experiments, mussel movements resulting in a higher final position on the plate were more common than the opposite (Table 1). Only in the temperature experiment at 22°C were downwards translocations more common than those upwards. None of these

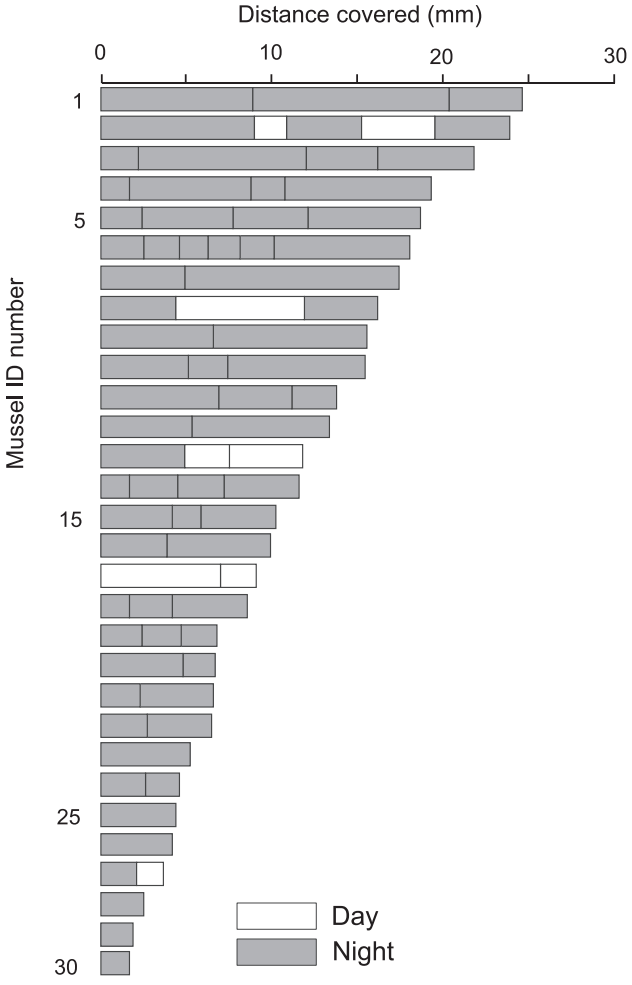

Figure 5. Translocations of all mussels that moved in a five-day experiment under exposure to a natural day–night cycle (Figure 1E). Each bar represents one animal. Superimposed bars denote discrete displacements recorded at 1 h intervals (not necessarily consecutive). Immobility periods are omitted.

differences were statistically significant when contrasted in a pairwise manner (ie within the same experimental setting); but the comparison across settings (ie the proportions of all upwards *vs* all downwards displacements, as detailed in Table 1) was significantly different (*t*-test, $p = 0.018$).

Behavioral differences between consecutive days

According to the RMA analyses, in all tests experimental mussels behaved similarly throughout each experimental period. On the other hand, GLM suggested time-related differences for two settings: permanent light and underside (Table 2). Pairwise contrasts (Fisher's least significant differences) indicated that in the underside experiment, the proportions of moving animals were significantly higher on day 8 than on days 1 and 4

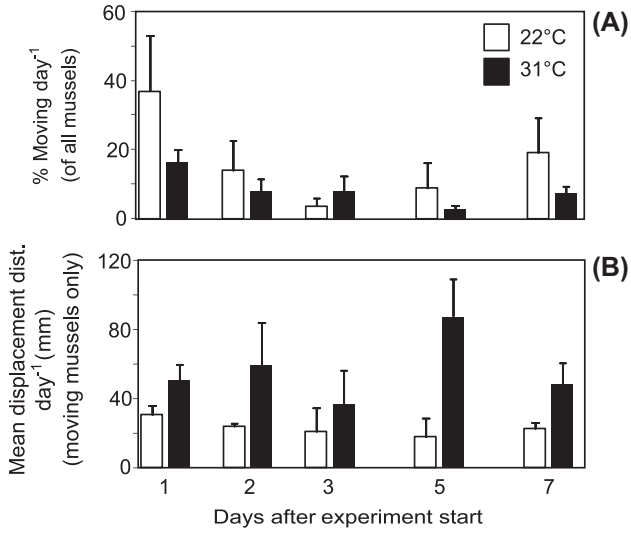

Figure 6. Daily proportions of translocating mussels (A) and mean daily distance covered by moving mussels (B) at 22°C and 31°C (means ± SE).

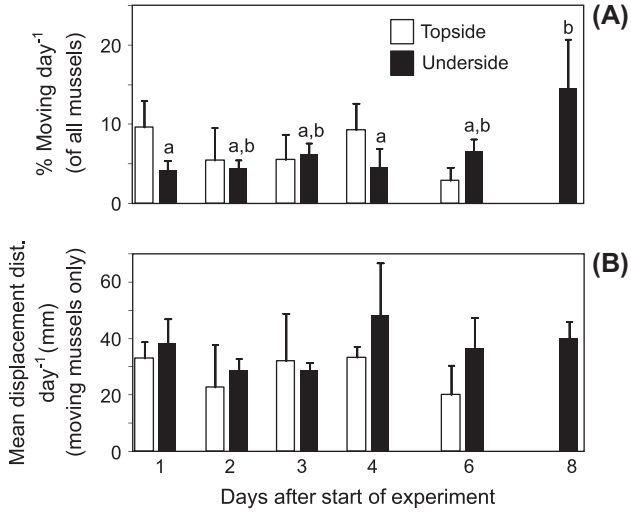

Figure 7. Daily proportions of translocating mussels (A) and mean daily distance covered by moving mussels (B) on the topside and the underside of experimental plates (mean ± SE). Different letters denote significant differences ($p < 0.05$, GLM and Fisher's LSD contrasts).

(Figure 7). The contrast in the proportions of moving animals under permanently illuminated conditions indicated significantly higher mobility on day 8 than during the previous 7 d (Figure 2).

Discussion

The fact that after having been forcibly dislodged, byssate mussels crawl and reattach has been described for several marine and freshwater species (Senawong

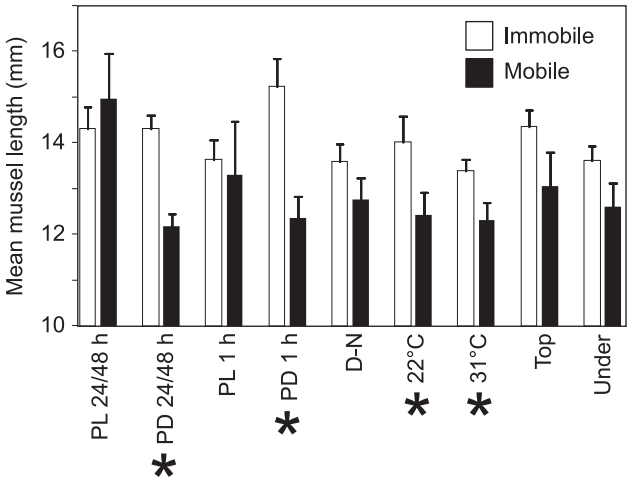

Figure 8. Mean lengths (±SE) of all immobile and mobile mussels in each experiment. PL 24/48 h and PD 24/48 h: permanent light/darkness, records every 24/48 h, all replicates pooled (Figure 1A and B); PL 1 h and PD 1 h: permanent light/darkness, records every 1 h (Figure 1C and D); D-N: day–night cycle (Figure 1E); 22°C and 31°C: tests at 22°C and 31°C, all replicates pooled (Figure 1F and G); Top and Under: topside and underside, all replicates pooled (Figure 1H and I). Asterisks denote differences significant at $p < 0.05$ (t-tests).

1970; Iwasaki 1997; Toomey et al. 2002; Kobak & Nowacki 2007; Iwasaki 2015), and the ability of already settled individuals to voluntarily detach, displace, and reattach elsewhere has been mentioned occasionally (Mackie et al. 1989; Cawein 1993; Eckroat et al. 1993), but little has been systematically investigated. Proactive antifouling treatments target mussel larvae, which are more sensitive than settled individuals, and therefore require lower doses of molluscicide (Claudi & Oliveira 2015). However, while proactive strategies assume that the only source of fouling are planktonic mussel larvae, the present results suggest that, in the case of *L. fortunei*, fouling of plant components can also result from juveniles and adults voluntarily detaching upstream and reattaching within the plant components. Of 879 firmly attached experimental animals assessed, 273 (31%) translocated at least once in the course of the five- to eight-day experiments. The proportions of mobile mussels varied widely, from ~16 to 53% (Table 1). The mean daily displacement distances covered by moving mussels were ~5–30 mm (Table 1), yet some animals moved >200 mm in one day. For the purposes of antifouling studies, detachment *per se* is more important than distance covered because voluntarily detached animals can be picked up by currents and travel longer distances before reattaching than those that translocate by crawling only.

All the results under different illumination settings indicate that light has a strong inhibiting effect on mussel detachment and translocation. In permanently darkened tanks, the proportions of translocating mussels were ~2.5 times higher than in the illuminated tanks. The distances covered were about twice as long in the dark, but the statistical significance of this difference is uncertain (Figures 2 and 3; Table 1). The additional experiment under permanently illuminated and permanently dark conditions, with records every 1 h (Figure 1C and D), also showed that mussels move much more actively in the dark (Figure 4). Furthermore, when exposed to a natural day–night cycle, almost six times more mussels moved at night than during the day (Figure 5; Table 1). The fact that these differences are due to light, rather than the result of circadian rhythms, is of particular importance because industrial plant components most sensitive to fouling by the mussel are in permanently dark areas.

The sensitivity of specimens of *L. fortunei* to light and strong negative phototaxis has been described in the past (Uryu et al. 1996; Iwasaki 2015; Morton 2015), but the effects of light on voluntary detachment, translocation, and reattachment have hitherto not been reported. No photosensory organs have been described in the golden mussel, although Morton (2015) suggested that the sensory papillae discovered on its siphonal septum, absent in other mytiloids, may be photosensory, which might account for the fact that, when illuminated, a passing hand shadow results in the siphons being partially retracted and valves shut slightly in up to >40% of individuals.

The byssate mode of life in densely packed mussel beds confers the animals with added protection from predators, currents and wave action (Bertness & Grosholz 1985; Okamura 1986; Walters & Wethey 1996; Côté & Jelnikar 1999; Sardiña et al. 2008). On the other hand, gregariousness may lead to cannibalism of larvae (MacIsaac et al. 1991) and higher competition pressure for food and space (Chase & Bailey 1996), and individuals buried deep in the colony may be affected by low oxygen concentrations and high concentrations of waste products (Burks et al. 2002). Thus, mussels that originally chose a location that was not advantageous or changed in fitness over time will detach and attempt to find a better site. Higher detachment and mobility rates in the dark than when illuminated are most probably a defensive strategy, since while unattached and crawling in search for a better site animals are more vulnerable to visual predators than when bysally attached. This vulnerability would obviously be higher in clear waters, like those typical for the marine ancestors of the golden mussel. In the freshwater bodies inhabited by *L. fortunei* transparency is usually much lower (in the Río de la Plata basin, typical Secchi disk depths are around 10–15 cm), which suggests that this behavior, inherited from its marine ancestors, is less effective in freshwater.

However, golden mussels also inhabit lentic waterbodies where transparency is considerably higher, with Secchi disk depths around 2–6 m, like the Río Tercero Reservoir, Argentina (Boltovskoy et al. 2009), and Lake Biwa, Japan (Terrel et al. 2012), where the adaptive advantage of not moving during the day is probably significant. It has also been suggested that lower mobility may be a response to the fact that mussels slow their physiological processes in order to lower emissions of metabolites that disclose their location to predators (Czarnoleski et al. 2010, 2011); yet the relationships between this mechanism and illumination are unclear.

Evidence on the effects of light on mobility from other byssate, freshwater mussels is mixed. For example, Korgina (1982) concluded that *Dreissena polymorpha* moves more and faster at night than during the day, but other studies found no association between light and movement (Toomey et al. 2002) or byssogenesis (Grutters et al. 2012). Kobak and Rynska (2014) concluded that, in the presence of crushed conspecifics (an alarm signal), illuminated mussels moved over longer distances than in darkness. However, most studies on *D. polymorpha* and *L. fortunei* agree that mussels move away from a light source (Korgina 1982; Iwasaki 1997; Toomey et al. 2002; Kobak & Nowacki 2007; Iwasaki 2015).

One interesting observation was that the translocation-inhibiting effects of light seemed to decrease over time when lighting conditions were stable. For example, in the permanent illumination experiment with records every 24/48 h (Figure 1A), the proportions of mobile mussels increased steadily from day 1 through day 8 (although significantly higher proportions of moving animals were observed only on day 8 with respect to the preceding week, GLM; Table 2 and Figure 2). A roughly similar trend was observed in the permanently illuminated experiment with records at hourly intervals (Figure 1C), where the proportions of mobile mussels increased from 1–6% on days 1–4 to 9–13% on days 5 and 6. This behavior suggests that in the light animals avoid detaching and moving around, but if adequate conditions (ie darkness) do not occur in a 'reasonable' amount of time, the urge to move overbears the risk of detaching and translocating in the light. Some previous experiments have shown the opposite trend, ie that mobility tends to decrease (rather than increase) over the duration of the experiments (Côté & Jelnikar 1999), but these results were based on manually dislodged animals before they reattach, rather than on bysally attached and voluntarily detaching individuals.

As opposed to light, no influence of temperature on mussel mobility could be found (Figure 6). The proportions of mobile mussels were higher at 22°C, but displacement distances were lower (Figure 6), and neither the proportions nor the displacement distances were significantly different at the two temperatures assayed (as reported previously for *D. polymorpha* [Toomey et al. 2002]). This conclusion, however, is preliminary because the effect-size of temperature on mussel mobility is very low and, therefore, low numbers of replicates yield marginal *p*-values (especially for displacement distance, see Table 2). Furthermore, while the present tests were performed at 22°C and 31°C, minimum water temperatures at which *L. fortunei* can survive are around 0°C (Karatayev et al. 2015), and a broader experimental temperature range may have yielded greater differences.

Similarly, the orientation of the substratum did not seem to show any consistent trend (Figure 7). Contrary to expectation, where mussels on the topside of the substratum may have been expected to move more actively because siltation is highest there, and because the risk of falling off (potentially into the mud) is higher on the underside, neither the proportions of moving animals, nor the distance covered were different between the two surfaces. The *p*-values in both the RMA and the GLM analyses were non-significant (Table 2) but, again, the very low effect-size of substratum orientation on mussel mobility (Table 2) indicates that higher numbers of replicates are necessary to validate this result. Colonization by planktonic larvae has been reported to occur preferentially on the underside of available substrata, presumably because such sites help to prevent predation, desiccation and dislodgement (Morton 1977), but translocating adults do not seem to behave alike. For zebra mussels, a preference for both the upper and the lower surface of substrata has been reported (Walz 1973; Marsden & Lansky 2000; Lewandowski 2001).

Although the experimental settings were not designed with a view to investigate the effects of mussel size on mobility (>90% of the animals used were 8–19 mm in length), mobile mussels were almost invariably (and often significantly) smaller than non-mobile ones (Figure 8; Table 1). In only one (of nine) comparisons were mobile animals slightly larger (mean: 15 mm) than those that never moved (14.1 mm), but this difference was not statistically significant ($p = 0.569$; Table 1). Unlike *D. polymorpha*, adult golden mussels do not build multi-layered beds, as most tend to be at least partly attached to the substratum (rather than to other shells only). On the other hand, juveniles up to 3–4 mm in length are often attached only to the sides of larger conspecifics, which indicates that they eventually detach and migrate deeper into the mussel bed (Correa et al. 2015). Higher mobility in smaller individuals is widespread among byssate mussels (Korgina 1982; Uryu et al. 1996; Côté & Jelnikar 1999; Burks et al. 2002; Kobak & Rynska 2014), which likely reflects changes in the fitness of the original settlement site as the animals grow (Cawein 1993).

While the data obtained in this survey consistently point at the ability of the golden mussel to voluntarily

detach, crawl, and reattach elsewhere, extrapolation of these results to conditions in the field has some caveats. In all the tests, mussels had been recently (one week earlier) transplanted to the experimental substrata. Thus, the threads they secreted might have been different from those they had before being removed from their original location (Eckroat et al. 1993), and their activity in terms of searching for a better site might have been higher than in nature.

Another potentially important difference between the present experimental design and environmental conditions in rivers, and especially in industrial plant installations, is that all tests were conducted under static conditions. Thus, it remains to be investigated whether the frequency of detachment in flowing waters is similar to that observed in the present experimental tanks (Clarke & Mc Mahon 1996). Statistical data suggest that the influence of light, which showed a high effect-size, might also hold in flowing waters, but those of temperature and substratum orientation need further investigation.

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