ORIGINAL ARTICLE

Parity and disparity between two *Chama* oysters: the reproductive biology of the Indo-Pacific *C. pacifica* Broderip, invasive to the Mediterranean Sea; and *C. savignyi* Lamy, indigenous to the Red Sea

Dror Zurel[1], Uri Gophna[2] & Yehuda Benayahu[3]

1 Porter School for Environmental Studies, Tel-Aviv University, Ramat-Aviv, Tel-Aviv, Israel
2 Department of Molecular Microbiology and Biotechnology, Tel-Aviv University, Ramat-Aviv, Tel-Aviv, Israel
3 Department of Zoology, Tel-Aviv University, Ramat-Aviv, Tel-Aviv, Israel

Abstract

The Indo-Pacific oyster *Chama pacifica* Broderip, 1835 (Mollusca, Bivalvia, Chamidae) is rarely found in the Northern Red Sea reefs of Eilat (Gulf of Aqaba), where it is outnumbered by its indigenous congener, *Chama savignyi* Lamy, 1921. The influx of Eritrean biota from the Red Sea into the Mediterranean Sea via the Suez Canal has led to the formation of massive *Chama* oyster beds along the Eastern Mediterranean shore. However, unlike the Northern Red Sea, the Eastern Mediterranean *Chama* beds are dominated by *C. pacifica* oysters, whereas *C. savignyi* is absent from this region. In an attempt to understand this difference in their respective distribution, the reproductive biology of both species was compared. Histological analysis of the male and female gonads, monitored monthly from March 2009 to August 2010 in both regions, revealed a similar reproductive cycle, comprising six stages: onset of gametogenesis, advanced gametogenesis, ripe, ready to spawn, spent and sexual rest. Female gonads demonstrated an additional, seventh stage – restoration, coinciding with inferred spawning of ripe gametes. Both species were found to be dioecious spawners, with a single, annual, temperature-dependent inferred spawning period. *Chama pacifica* was found to reproduce efficiently in maximal Mediterranean seawater temperatures not experienced by the Northern Red Sea *Chama* populations. This study demonstrates the high invasive potential of an oyster species despite its rarity in its source region.

Introduction

Members of the oyster family Chamidae are common in Indo-Pacific subtidal habitats, adhering to the sides of rocks, where they are usually well hidden by a fine growth of algae that extends equally over the rocks and the oyster shells (Purchon 1968). Chamidae species are known for their efficient filter-feeding abilities and thus may compete with other benthic filter-feeders for both space and food (Raj 2008). In Eilat (Northern Gulf of Aqaba, Red Sea), *Chama savignyi* Lamy, 1921, is quite abundant in

reefal habitats, growing among coral colonies (Fig. 1A), from shallow subtidal rocky formations down to ∼20 m. *Chama savignyi* outnumbers all other oyster species there. However, its congener, *Chama pacifica* Broderip, 1835, is seldom found in Eilat (see Results).

The opening of the Suez Canal in 1869 connected the Eastern Mediterranean Sea and the Gulf of Suez (Northern Red Sea), linking two biogeographic provinces, the Atlanto-Mediterranean and the Indo-Pacific (Spanier & Galil 1991). This triggered an invasion of Indo-Pacific (Erythrean) biota into the Mediterranean Sea (termed the
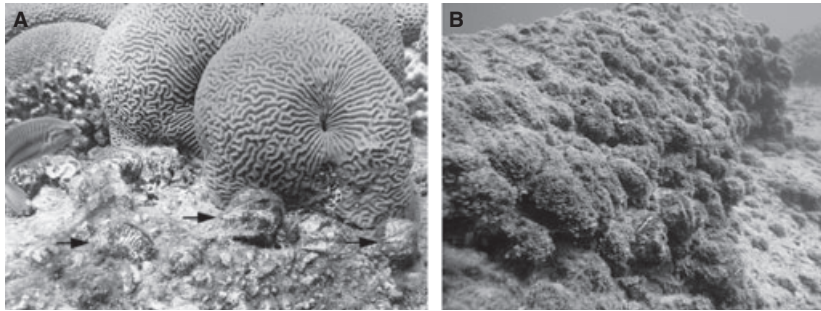
Fig. 1. (A) *Chama savignyi* oysters on a Northern Red Sea coral reef. (B) *C. pacifica* oyster bed on an Eastern Mediterranean rock formation.

'Lessepsian migration'; Por 1978), imposing changes on the Mediterranean food web (Argyrou *et al.* 1999; Rilov *et al.* 2002), generating constant competitive stress on the local fauna (Fishelson *et al.* 2002; Atad 2005), and also introducing alien parasites (Diamant 1998). *Chama pacifica* was first recorded in the Mediterranean from Alexandria, Egypt (Tillier & Bavay 1905) and a few decades later from Lebanon, Turkey, and Cyprus (Galil 2008). In contrast to its rarity in the Eilat reefs, *C. pacifica* has formed dense populations in the form of gregarious beds off the Israeli Mediterranean coast (see Results below; Fig. 1B), where it has almost completely replaced *Chama gryphoides* (Linnaeus, 1758), its indigenous Mediterranean congener (Galil 2008). On the other hand, *C. savignyi* has not yet been recorded in the Mediterranean Sea, despite the steady invasion of the Red Sea biota into the region (Zenetos *et al.* 2010).

Reproductive traits are considered among the most significant factors determining the success of a bivalve species in establishing a population in a new environment (reviewed by Vermeij 1996). In Chile, for example, the oyster *Crassostrea gigas*, despite having been introduced into several habitats, has only succeeded in maintaining a growing population in environments in which the temperature of the seawater has allowed optimal development of the female gonads (Castaños *et al.* 2009). The freshwater mussels *Limnoperna fortunei* and *Dreissena polymorpha* (family Mytilidae) have both successfully invaded freshwater bodies in East Asian countries, such as China and Japan (Morton 1975; Nakai 1995), and South American ones, such as Argentina and Bolivia (Pastorino *et al.* 1993). *Limnoperna fortunei* was found to have greater invasive potential than *D. polymorpha*, partially due to its longer reproductive season (Karatayev *et al.* 2007). The higher reproductive ability of the invasive mussel *Mytilus galloprovincialis* has been suggested to contribute to its successful competition with the indigenous *Perna perna* along the South African shores (Zardi *et al.* 2007).

The reasons for the disparity in occurrence of the two congeners *C. pacifica* and *C. savignyi* in both the Eastern Mediterranean Sea and Eilat are still not known.

A comparison of their reproductive traits could shed light on the factors that have led to this pattern. However, such a study requires examination of a sufficient number of oysters and, as *C. pacifica* is extremely rare in Eilat and *C. savignyi* is absent from the Eastern Mediterranean Sea, a comparison is not feasible. Conversely, the large population of *C. pacifica* in the latter region provides a sufficient sample of the animal for examination of its reproductive traits and it can be compared with the Red Sea *C. savignyi*. The present study quantifies the abundance of *C. pacifica* and *C. savignyi* in the Eastern Mediterranean Sea and Eilat and examines for the first time their annual gonad development and reproductive cycles. During an 18-month period, animals of the two species were examined histologically and their gonad development monitored, in an attempt to understand the disparity in their abundance in the respective regions (Eastern Mediterranean Sea *versus* Northern Red Sea). The current study addressed the following questions: first, is the difference in the abundance of the two chamid species in Eilat due to certain species-specific reproductive traits; and second, have certain reproductive traits led to the successful establishment of *C. pacifica* in the Eastern Mediterranean Sea? The study also provides for the first time, quantitative data regarding the abundance and density of both oyster species in the two regions.

Material and Methods

Study sites and oyster abundance

Chama pacifica was collected from Sdot Yam (29º 32′28″ N, 53º 34′10″ E), along the Israeli Eastern Mediterranean Sea (EMS); and *Chama savignyi* from opposite the Interuniversity Institute of Marine Science (IUI) (30º 29′06″ N, 55 º 34′03″ E), Eilat, at 2–5 and 1–2 m depth, respectively. The density of the two species was determined by counting specimens in the respective regions (5 m depth), using 1-m^2 quadrats (n = 10 random counts in each region, approximately 20 m apart, April–May 2009).

Sampling and histological studies

During March 2009–August 2010, monthly samples of 10 randomly collected specimens of each species were obtained from the largest size group (shell length >10 cm) in each region. Due to bad weather conditions, no sampling was conducted in April 2009 and January 2010 in either region. In March and July 2009 and February 2010, the EMS sample comprised 20 *Chama pacifica* oysters. The ambient seawater temperature was recorded on the day of collection using a Vyper diving computer (Suunto, Vantaa, Finland). The oysters were removed from the substrate with a hammer and chisel, kept in seawater and immediately dissected: the Sdot Yam specimens at Tel Aviv University (TAU) and the Eilat ones at the IUI. They were opened with a hammer and chisel, and the soft body was separated from the shell and individually placed overnight in 50-ml PVC containers with 4% formaldehyde in seawater, then rinsed with distilled water, transferred to 70% ethanol and stored at 4 °C.

The gonads of *Chama pacifica* and *C. savignyi* surround the surface of the gut (see Yonge, 1966). Following fixation (see above), a random slice of the gonad (~10 × 20 mm) was removed from each oyster with a scalpel for preparation of histological sections. After dehydration in a graded series of ethanol and butanol and embedding in paraffin, serial sections, 8 μm thick, were prepared using an MIR microtome (Thermo Fisher Scientific, Waltham, MA, USA) and stained with Delafield hematoxylin-eosin. Fifteen sections were prepared from each oyster and were examined after mounting with an Optiphot Nikon microscope (×400) attached to a Nikon HB 10101 AF Camera. The presence of gonads and their sex were determined in each section. To measure oocyte size, five oocytes from each female oyster were randomly chosen and the size of their longest axis crossing the nucleus (maximum diameter, following Lango-Reynoso *et al.* 2000 and Castaños *et al.* 2009) was measured to the nearest μm, using the measuring tool of the Nikon Camera (see above). The developmental stages of the male and female gonads were determined following the seven-stage characterization defined by Darriba *et al.* (2004) for the study of the razor clam *Ensis arcuatus* for both sexes, as follows:

- *Start of gametogenesis*: characterized by the presence of a few follicles within the gonads, in which gametes in meiotic stages are found on the follicle wall.
- *Early and advanced gametogenesis*: the size of the follicles increases and they occupy a larger percentage of the gonad. In males the center of the follicles contains a mass of spermatozoa, while immature sperm cells develop along the follicle wall. In females, free oocytes are found inside the lumen of the follicle.
- *Ripe*: follicles densely packed with gametes occupy the entire space of the gonad.
- *Spawning*: gonoducts appear full of gametes; follicles lose their packed image and many follicular walls are ruptured.
- *Restoration*: follicles are condensed, allowing immature gametes to mature and consequent further spawning.
- *Spent (exhaustion)*: follicles decrease in size and are almost empty; a preceding spawning event can thus be inferred from their presence. At this stage, tissue appears between the follicles and the latter reveal an empty lumen, occasionally with a few individual ripe gametes.
- *Sexual rest*: follicles and gametes are not found and most of the gonad is occupied by collagen tissue and fibroblasts.

Results

Oyster abundance

At Sdot Yam, beds of *Chama pacifica* were commonly found on the aeolinite sandstone formations known as Kurkar sandstone (Fishelson *et al.* 2002) at a wide depth range (1–25 m), with a density of 13.6 ± 5.3 m^{-2} at 5 m. They form dense aggregations, often growing next to another Lessepsian invasive oyster, *Spondylus spinosus*, as previously described by Mienis *et al.* (1993a). Such oyster beds are rather new to the Eastern Mediterranean Sea and have been noticed only in the last few decades since *C. pacifica* invaded the region (Mienis *et al.* 1993b). In Eilat, this latter species was found in the current survey to be extremely rare and only two or three oysters could be found during a 60-min dive. However, at 5 m, *Chama savignyi* exhibited a density of 5.4 ± 2.7 oysters m^{-2}, although it was never found in Sdot Yam or in any other Eastern Mediterranean Sea site explored by us (pers. obs.). Both species were found in Eilat at depths of up to 20 m. The oyster density in Sdot Yam is significantly higher than in Eilat (*C. pacifica versus C. savignyi*, paired *t*-test $t = 5.32$, P-value <0.05, n = 20).

Histological studies and reproductive stages

Both *C. pacifica* and *C. savignyi* were found to be dioecious and no hermaphrodites were found in any of the oysters examined. Their reproductive stages were rather similar and both corresponded to those assigned by Darriba *et al.* (2004) (see above). At the start of spermatogenesis, some follicles were present in the gonads, along with a few dispersed spermatocytes attached to the follicular wall (Fig. 2A: *C. pacifica*; Fig. 3A: *C. savignyi*). Early
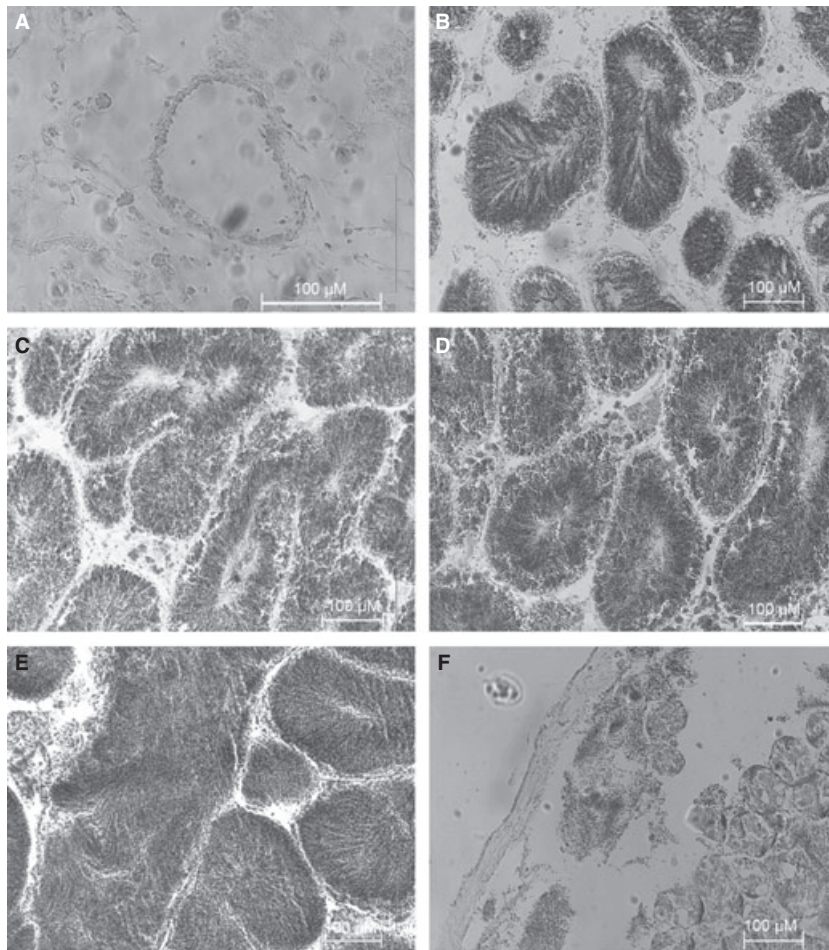
Fig. 2. *Chama pacifica*, Sdot Yam, Israeli Eastern Mediterranean Sea. Histological sections showing reproductive stages of males. (A) Start of spermatogenesis. (B) Early spermatogenesis. (C) Advanced spermatogenesis. (D) Ripe spermaries. (E) Ready to spawn spermaries. (F) Spent spermaries.

spermatogenesis was recorded in *C. pacifica* (Fig. 2B) but not in *C. savignyi,* most probably due to lack of sampling in January and February 2010 (see Material and Methods). Advanced spermatogenesis was recorded for both species (Fig. 2C: *C. pacifica*; Fig. 3B: *C. savignyi*), as well as ripe (Fig. 2D: *C. pacifica*; Fig. 3C: *C. savignyi*), spawning (Fig. 2E: *C. pacifica*; Fig. 3D: *C. savignyi*) as well as spent spermaries (Fig. 2F: *C. pacifica*; Fig. 3E: *C. savignyi*).

Among the females, onset of oogenesis was found in both *Chama* species (Fig. 4A: *C. pacifica*; Fig. 5A: *C. savignyi*), as well as the advanced oogenesis stage (Fig. 4B: *C. pacifica*; Fig. 5B: *C. savignyi*), ripe gonads (Fig. 4C: *C. pacifica*; Fig. 5C: *C. savignyi*), and spawning (Fig. 4D: *C. pacifica*; Fig. 5D: *C. savignyi*). The restoration stage was observed during the inferred spawning period of both species. Condensed follicles featuring immature oocytes were also found concurrently at that stage with the ruptured follicles post-spawning (Fig. 4E: *C. pacifica*; Fig. 5E: *C. savignyi*). Spent gonads (Fig. 4F: *C. pacifica*; Fig. 5F: *C. savignyi*) and sexual rest (Fig. 4G: *C. pacifica*; Fig. 3F: *C. savignyi*) were found in both species. No follicles were

seen in the gonad tissue during sexual rest and therefore the sex of such oysters could not be determined.

Annual gonad development and reproductive cycle

Over the course of the study, in any given month, both *Chama pacifica* and *Chama savignyi* exhibited at least one reproductive stage (Table 1). On the first sampling date, March 2009, *C. pacifica* featured onset of gametogenesis, whereas *C. savignyi* was in the spawning stage. During March–August 2009, 61.5% of the males of *C. savignyi* were either at the ripe, spawning or restoration stages, whereas the rest were undergoing either onset (11.5%) or early (15%) spermatogenesis or had spent gonads (8%) (Table 1). During this period, 80% of the females were in the ripe, spawning or restoration stages, the rest undergoing onset of gametogenesis (Table 1).

Based on histological sections, spawning of *C. pacifica* males was inferred to have begun in May 2009, and the females demonstrated the onset and early gametogenesis stages during the same period. During May–August
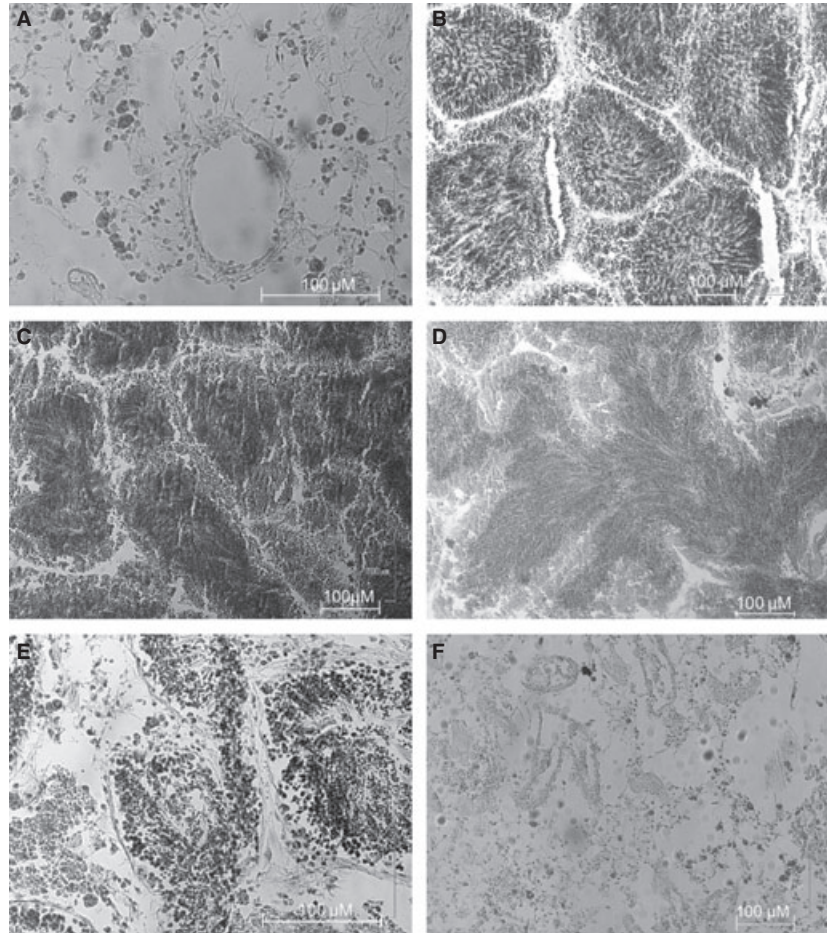
Fig. 3. *Chama savignyi*, Eilat, Northern Gulf of Aqaba. Histological sections showing reproductive stages of males. (A) Onset of spermatogenesis. (B) Advanced spermatogenesis. (C) Ripe spermaries. (D) Ready to spawn spermaries. (E) Spent spermaries. (F) Sexual rest.

2009, 83% of the males were ripe, spawning or in the restoration stage, and the remaining 17% had spent gonads. Most of the females at that time (48%) featured ripe, spawning or restoration stages. Onset of oogenesis (38%), advanced oogenesis (7%), and spent (7%) stages were also found in females at that time. In September 2009, both *Chama* species featured advanced oogenesis, although spawning could not be inferred from the histological examination. Sexual rest was found in *C. pacifica* in September and October 2009 and in *C. savignyi* during September–December 2009. In both species, sexual rest coincided with gametogenesis. During September 2009–April 2010, all male and female *C. pacifica* were either at onset of gametogenesis (54 and 63%, respectively), or at early or advanced gametogenesis (51 and 37%, respectively). During September–December 2009, 75% of the males of *C. savignyi* and all the females featured onset of gametogenesis, whereas early or advanced gametogenesis was noted in only 25% of the males. In March 2010, spawning was inferred in *C. savignyi*. From then until August 2010, 67% of males and 65% of females were ripe, spawning

or at the restoration stage. The remaining males (33%) and 4% of the females featured advanced gametogenesis, while the rest of the females (30%) had spent gonads. Spawning was inferred for *C. pacifica* in May 2010 and from then until August 2010, 67% of the males and 62% of the females were ripe, spawning or at the restoration stage. The remaining male and female oysters (33 and 38%, respectively) were at the advanced gametogenesis stage.

Examination of all *C. pacifica* oysters containing gonads revealed a sex ratio of 59 males (40.4%) to 87 females (59.6%) with no significant difference between the occurrence of each sex in the samples ($\chi^2 = 3.3$, P = 0.07). The sex ratio of *C. savignyi* was 65 males (55.2%) to 53 females (44.8%), and similarly indicated no significant difference ($\chi^2 = 0.84$, P = 0.36). Sdot Yam *C. pacifica* showed a significant tendency toward females when compared to Red Sea *C. savignyi*, ($\chi^2 = 5.9$, P = 0.015).

Figure 6 presents the average maximal diameter (± SD) of the oocytes of *C. pacifica* and *C. savignyi* throughout the study. This diameter in both species corresponded
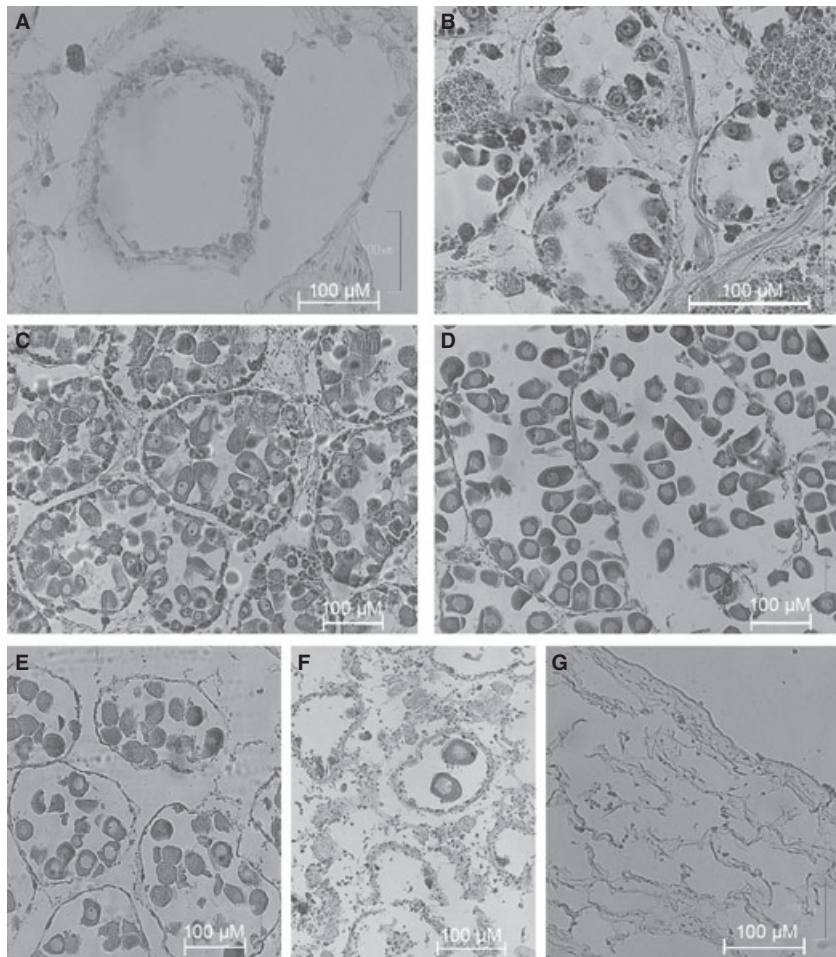
Fig. 4. *Chama pacifica*, Sdot Yam, Israeli Eastern Mediterranean Sea. Histological sections showing reproductive stages of females. (A) Start of oogenesis. (B) Advanced oogenesis. (C) Ripe oocytes. (D) Ready to spawn oocytes. (E) Restoration of immature oocytes for further spawning. (F) Spent gonads. (G) Sexual rest.

with the ambient seawater temperature curve, *i.e.* larger oocytes occurred when temperature was high and *vice versa*.

Significant differences were recognized among the average oocyte diameter of both oyster species in the different months (Kruskal–Wallis test *C. pacifica*: H = 62.8, P < 0.01; *C. savignyi*: H = 36.61, P < 0.01). In *C. pacifica*, the maximal average diameter of oocytes did not differ significantly between the two monitored spring–summer periods (May–August 2009 and 2010) (Mann–Whitney test, P = 0.06, t = 73.5, n = 22 for each period). However, significant differences were found between the spring–summer periods of both years (May–August 2009 and 2010) and the autumn–winter periods (October 2009–March 2010, n = 25) (Mann–Whitney test, t = 0, P = 2.7E-08; t = 0, P = 4.2E-05, respectively). In *C. savignyi*, the average maximal diameter of oocytes did not differ significantly between the two monitored spring-summer periods (March–August 2009 and 2010) (Mann–Whitney test, P = 0.12, t = 32, n = 20 and 22, respectively). However, significant differences between the

average maximal oocyte diameters were found when each of the spring–summer periods (May–August 2009 and 2010) was compared to the autumn–winter period (September–December 2009, n = 20) (Mann–Whitney tests: t = 0, P = 0.001 and t = 0, P = 1.45E-05, respectively). In both oyster species the high standard deviation around the average values in July 2009 reflected the co-occurrence of immature and mature oocytes at the restoration stage (Figs 5E and 6E).

The largest oocyte diameter of *C. pacifica* (85.5 μm) was found in August 2009 and was smaller than that of the largest *C. savignyi* oocyte (98.9 μm), found in May 2010. Notably, the maximal average diameter of the five largest mature oocytes measured in ripe and spawning oogenic stages did not differ significantly between the two species (paired t-test P > 0.05). Mann–Whitney pairwise comparisons revealed significant differences in maximal oocyte diameter between start, early and advanced oogenic stages. No significant differences in maximal oocyte diameter were found between advanced, ripe and spawning stages (Table 2). Comparisons were applied to
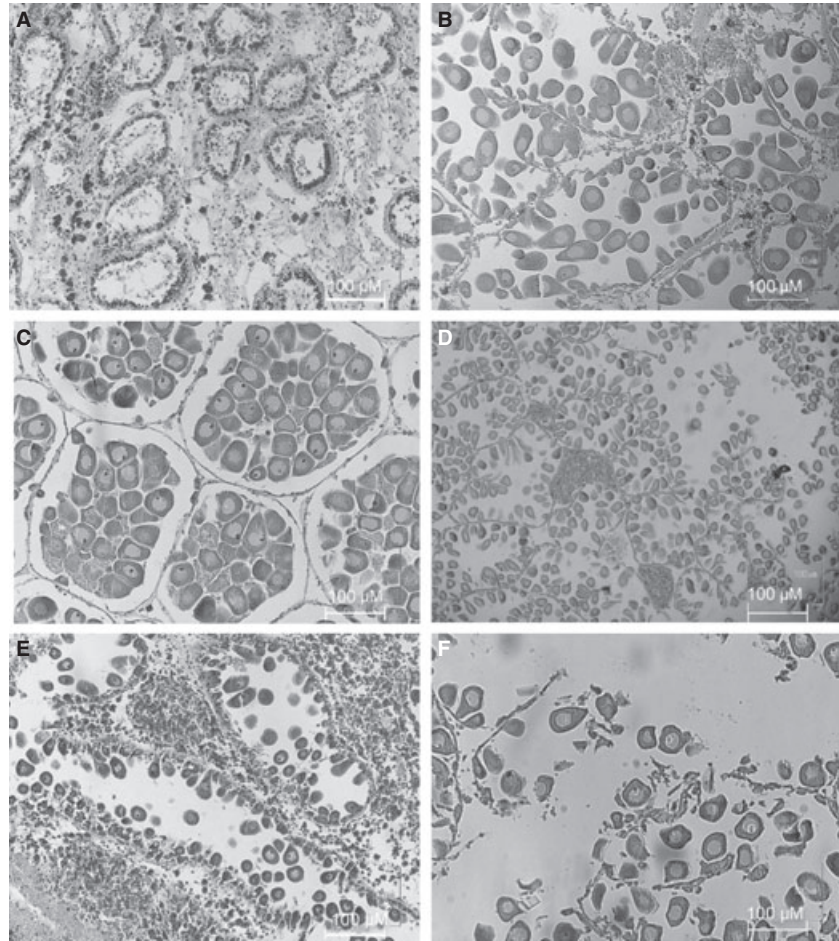
Fig. 5. *Chama savignyi*, Eilat, Northern Gulf of Aqaba. Histological sections showing reproductive stages of females. (A) Onset of gametogenesis. (B) Advanced oogenesis. (C) Ripe oovaries. (D) Ready to spawn oocytes. (E) Restoration of immature oocytes for further spawning. (F) Spent gonads.

pooled data from both 2009 and 2010 samples. Similar results were obtained for *C. savignyi* (Table 3).

Discussion

The present study compared the abundance and reproductive traits of two Indo-Pacific congeneric oysters, *Chama pacifica* and *Chama savignyi*. Both the invasive *C. pacifica* in Sdot Yam and the indigenous, congeneric *C. savignyi* in Eilat, are prominent in the respective regions. Our results reveal that *C. pacifica* has become a notable component of the shallow Eastern Mediterranean benthos, occasionally in association with *Spondylus spinosus*, another Lessepsian invasive oyster (see Results). The density of *C. pacifica* in Sdot Yam found in the current study, resembles that obtained at three other sites along the Eastern Mediterranean Israeli coast: the submerged rocky habitat off Tel Aviv (Zurel *et al.* unpublished data), steel pillars supporting the coal conveyer belt at Hadera powerplant, and the rocky shore at Shiqmona near Haifa (Sharon 2006). These two invasive oysters appear to be

among the most conspicuous solitary organisms on the Eastern Mediterranean subtidal hard substrate, mainly Kurkar sandstone formations.

Reproductive stages and gametogenic cycle

Prior to the current study, the only available data on reproduction of a chamid species were on *Chama congregate*, studied in North Carolina and found to be a dioecious spawner (Labarbera & Chanley 1971). The current study has revealed that *C. pacifica* and *C. savignyi* are also dioecious spawners, and are characterized by similar reproductive features, with an inferred spawning period throughout spring and summer (Fig. 6, Table 1). The gonad stages of the two oysters correspond to those previously found for the razor clam *Ensis arcuatus* (see Darriba *et al.* 2004) and the oyster *Crassostrea gigas* (Castaños *et al.* 2009). Restoration and recovery stages, deduced from the large standard deviation around the average oocyte diameter, measured during the inferred spawning period (Fig. 6), have been found in several other bivalves

Table 1. *Chama pacifica* (CP), Sdot Yam, Israeli Eastern Mediterranean coast, and *C. savignyi* (CS), Eilat, Northern Red Sea. Number of oysters: females (F), males (M) or unidentified sex (U) during the study with respect to reproductive stage (Ripe, Spawning and Restoration lumped together).

|	Sexual rest		Start of gametogenesis				Early and advanced gametogenesis				Ripe/spawning/restoration				Spent				Sum	
	U		F		M		F		M		F		M		F		M			
Month	CP	CS	CP	CS	CP	CS	CP	CS	CP	CS	CP	CS	CP	CS	CP	CS	CP	CS	CP	CS
March 2009			6		11					1		4		6					18	10
May 2009			5	1			2			2	6	3							10	10
July 2009				1						2	10	5	1					1	10	9
August 2009		4	1							1	4	1	1	1	2		1	2	6	10
September 2009	1	2		4	4	2	2	2	3										10	10
October 2009	1	3	1	4	6	1	2			2									10	10
November 2009		2	6	4	3	5				1									10	10
December 2009		1	7	4	2	4			1	1									10	10
February 2010			2		2		4		4										12	
March 2010	1		2		2		1	2	3	1		1		2				2	9	8
April 2010							6	4	4			2		1					10	7
May 2010							1			1	6	3	2	4				3	10	10
June 2010											7	3							10	
July 2010							3			1	5	5		5					9	10
August 2010										1	4	4	6	3				2	10	6
Sum	3	12	29	18	30	12	19	13	20	5	36	31	16	23	2		1	10	136	120
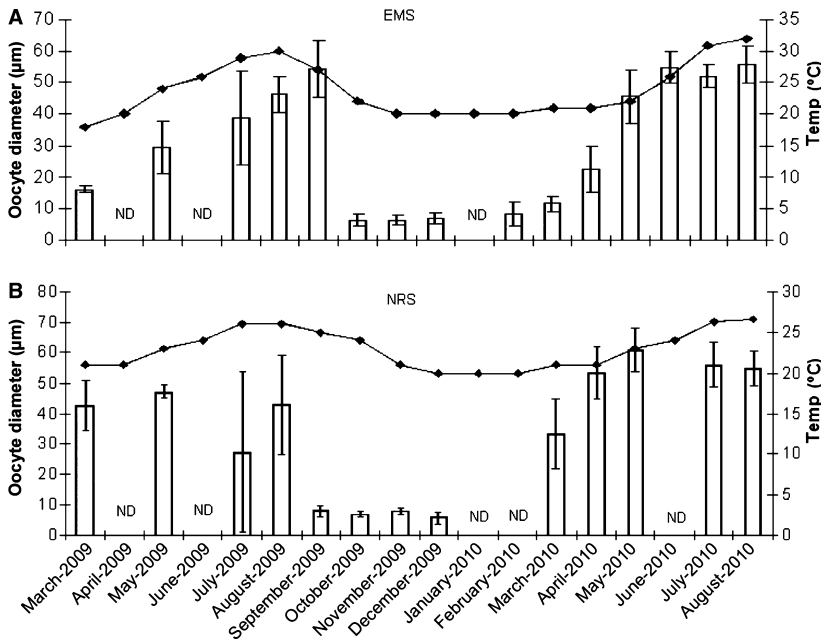
Fig. 6. Average maximal diameter of oocytes (± S.D., n = 15 oocytes per each month, ND = no data) and the ambient seawater temperature (°C) curve for *Chama pacifica* in Sdot Yam, Israeli Eastern Mediterranean Sea (A) and *C. savignyi* in Eilat, Northern Gulf of Aqaba (B).

that feature more than one spawning event per year, such as the South American scallops *Amusium laurenti* and *Amusium papyraceum* (see Penchaszadeh *et al.* 2000). We suggest, therefore, that the restoration stage may facilitate the prolonged spawning period found in *C. pacifica* and *C. savignyi* over the entire spring and summer, and thus also contributes to their success in the respective regions.

Spawning in oysters, such as *C. gigas*, has been found to be related to annual seawater temperature rise (Castaños *et al.* 2009). In our study, spawning was first inferred for both *Chama* species when seawater

Table 2. Whitney pairwise comparisons between maximal average oocyte diameters of *Chama pacifica* measured at different oogenesis stages.

	Start of oogenesis	Early oogenesis	Advanced oogenesis	Ripe	Spawning
Start of oogenesis		0.012	0.008	0.02	0.004
Early oogenesis			0.008	0.02	0.004
Advanced oogenesis				0.9	0.18
Ripe					0.11
Spawning					

Table 3. Mann–Whitney pairwise comparisons between maximal average oocyte diameters of *Chama savignyi* measured at different oogenic stages.

	Start of oogenesis	Advanced oogenesis	Ripe	Spawning
Start of oogenesis		0.023	0.023	0.006
Advanced oogenesis			0.66	0.04
Ripe				0.14
Spawning				

temperature was >21 °C (Table 1, Fig. 6). We found a 5-month inferred spawning period for the Sdot Yam *C. pacifica* (May–September) and a 6-month period for the Eilat *C. savignyi* (March–August). We suggest that the lower winter seawater temperature measured in Sdot Yam (14–18 °C) compared with Eilat (20–21 °C) has led to the differences in the length of the respective inferred spawning periods. Exposure of an invasive species to the temperature regime prevailing in its new environment may conduce to a change in the timing and duration of its spawning period, as occurred for example when *C. gigas* was introduced into France (Enríquez-Díaz *et al.* 2009). The invasive *C. pacifica* appears to have adapted to the Eastern Mediterranean Sea temperature regime, and its inferred spawning period commences two months after that of the Eilat *C. savignyi* (Table 1), thus meeting the temperature threshold of >21 °C (Table 1, Fig. 6). We suggest that under the Northern Red Sea conditions prevailing in Eilat, both Chamidae species are expected to show a similar reproductive cycle, with temperature-dependent spawning periods.

Disparity in abundance of *Chama savignyi* and *Chama pacifica*

Despite the general similarity in the reproduction of *C. pacifica* and *C. savignyi*, the former is scarce in Eilat, in opposition to its remarkably high abundance in Sdot Yam. Consequently, reproduction does not explain the rarity of *C. pacifica* in the Eilat reefs.

In a recent study on mollusk shells found along the Saudi Arabia coast, Central Red Sea (Wronski 2010), where the summer seawater temperature of 31 °C resembles that of the Eastern Mediterranean Sea (see Results), *C. pacifica* comprised >4% of the shells. Additionally, warm seawater temperatures were found to be favorable for the symbiotic gill bacteria which co-invaded the Eastern Mediterranean from the Red Sea along with *C. pacifica* (Zurel *et al.* 2011). We therefore suggest that the warmer seawater temperature prevailing in these two regions, compared to the typical summer temperature of Eilat (27 °C, see Genin 1995), has led to the proliferation of *C. pacifica*. However, the mechanism by which the highest temperature (31 ° *versus* 27 °C) may affect the oyster is not necessarily reproduction-related. The summer seawater temperature of the Eastern Mediterranean Sea contributed considerably to the successful invasion of *C. pacifica* of that region.

In the current study, the Sdot Yam *C. pacifica* population had a significantly higher proportion of females compared with Eilat *C. savignyi*. Such a tendency has been suggested to promote population increase, which may also conduce to a successful invasion (Galbreath *et al.* 2004). At present, invasive populations of *C. pacifica* are known from Israel (Mienis *et al.* 1993a), Cyprus (Zenetos *et al.* 2009) and Turkey (Türkmen *et al.* 2005). Recent findings of *C. pacifica* as far west as the Greek Aegean (Manousis *et al.* 2010) suggest an even further spread of this species in the region. It should be noted that both Sdot Yam minimum and maximum temperatures measured in our study during 2010 were 2 °C higher (20 °C and 32 °C) than in 2009 (18 °C and 30 °C). Moreover, the minimum temperature in Sdot Yam in 2010 was 4 °C higher than the 14 °C minimum known for the region (Galil & Zenetos 2002). These findings further demonstrate a pattern of temperature rise of the Mediterranean Sea (Galil 2007; Occhipinti-Ambrogi 2007; Klausmeyer & Shaw 2009). As *C. pacifica* was found to be able to contend with a wide temperature regime, the predicted scenarios of higher winter temperatures in the Mediterranean (Klausmeyer & Shaw 2009) are expected to expand the distribution of *C. pacifica* to regions beyond those known at present.

References

Argyrou M., Demetropoulos A., Hadjichristophorou M. (1999) Expansion of the macroalga *Caulerpa racemosa* and changes in softbottom macrofaunal assemblages in Moni Bay, Cyprus. *Oceanologica Acta*, **22**, 517–528.

Atad I. (2005) The limpet *Cellana rota* as a Lessepsian migrant model: arrival, establishment and competition with the Mediterranean limpet, *Patella caerulea*. MSc thesis, Tel Aviv University.

Castaños C., Pascual M., Camacho A.P. (2009) Reproductive biology of the nonnative oyster, *Crassostrea gigas* (Thunberg, 1793), as a key factor for its successful spread along the rocky shores of Northern Patagonia, Argentina. *Journal of Shellfish Research*, **28**, 837–847.

Darriba S., San Juan F., Guerra A. (2004) Reproductive cycle of the razor clam *Ensis arcuatus* (Jeffreys, 1865) in northwest Spain and its relation to environmental conditions. *Journal of Experimental Marine Biology and Ecology*, **311**, 101–115.

Diamant A. (1998) Red drum *Sciaenops ocellatus* (Sciaenidae), a recent introduction to Mediterranean mariculture, is susceptible to *Myxidium leei* (Myxosporea). *Aquaculture*, **162**, 33–39.

Enríquez-Díaz M., Pouvreau S., Chávez-Villalba J., Le Pennec M. (2009) Gametogenesis, reproductive investment, and spawning behavior of the Pacific giant oyster *Crassostrea gigas*: evidence of an environment-dependent strategy. *Aquaculture International*, **17**, 491–506.

Fishelson L., Bresler V., Abelson A., Stone L., Gefen E., Rosenfeld M., Mokady O. (2002) The two sides of man-induced changes in littoral marine communities: eastern Mediterranean and the Red Sea as an example. *Science of the Total Environment*, **296**, 139–151.

Galbreath J.G., Smith J.E., Terry R.S., Becnel J.J., Dunn A.M. (2004) Invasion success of *Fibrillanosema crangonycis*, n.sp., n.g.: a novel vertically transmitted microsporidian parasite from the invasive amphipod host *Crangonyx pseudogracilis*. *International Journal for Parasitology*, **34**, 235–244.

Galil B.S. (2007) Loss or gain? Invasive aliens and biodiversity in the Mediterranean Sea. *Marine Pollution Bulletin*, **55**, 314–322.

Galil B.S. (2008) Alien species in the Mediterranean Sea – which, when, where, why? *Hydrobiologia*, **606**, 105–116.

Galil B.S., Zenetos A. (2002) A sea change – Exotics in the Eastern Mediterranean. In: Leppäkoski E., Gollasch S.,

Ollenin S. (Eds), *Invasive aquatic species of Europe: distribution, impacts, and management*. Kluwer Academic Publishers, Dordrecht: 325–336.

Genin A., Lazar B., Brenner S. (1995) Vertical mixing and coral death in the Red Sea following the eruption of Mount Pinatubo. *Nature*, **377**, 507–510.

Karatayev A.Y., Boltovskoy D., Padilla D.K., Burlakova L.E. (2007) The invasive bivalves *Dreissena polymorpha* and *Limnoperna fortunei*: parallels, contrasts, potential spread and invasion impacts. *Journal of Shellfish Research*, **26**, 205–213.

Klausmeyer K.R., Shaw M.R. (2009) Climate Change, habitat loss, protected areas and the climate adaptation potential of species in Mediterranean ecosystems worldwide. *PLoS ONE*, **4**, e6392 doi: 10.1371/journal.pone.0006392.

Labarbera M., Chanley P. (1971) Larval and postlarval development of corrugated Jewel Box Clam *Chama congregata* Conrad (Bivalvia-Chamidae). *Bulletin of Marine Science*, **21**, 733–744.

Lango-Reynoso F., Chavez-Villalba J., Cochard J.C., Le Pennec M. (2000) Oocyte size, a means to evaluate the gametogenic development of the Pacific oyster, *Crassostrea gigas* (Thunberg). *Aquaculture*, **190**, 183–199.

Manousis T., Mpardakis G., Paraskevopoulos C., Galinou-Mitsoudi S. (2010) The Bivalvia Mollusca of Thessaloniki & Thermaikos Gulfs (North Aegean Sea, Greece) with emphasis on new species for Hellenic waters. *Journal of Biological Research-Thessaloniki*, **14**, 161–179.

Mienis H.K., Galili E., Rapoport J. (1993a) The spiny oyster, *Spondylus spinosus*, a well established Indo-Pacific bivalve in the eastern Mediterranean off Israel (Mollusca, Bivalvia, Spondylidae). *Zoology in the Middle East*, **9**, 83–91.

Mienis H.K., Galili E., Rapoport J. (1993b) On the presence of the Indo-Pacific bivalve *Chama pacifica* in the Eastern Mediterranean (Mollusca, Bivalvia, Chamidae). *Gloria Maris*, **32**, 13–18.

Morton B. (1975) The colonization of Hong Kong's raw water supply system by *Limnoperna fortunei* (Dunker, 1857) (Bivalvia: Mytilacea) from China. *Malacologia Review*, **8**, 91–105.

Nakai K. (1995) Intrusion of the freshwater mytilid mussel, *Limnoperna fortunei* (Dunker, 1857), into Japan. *Kansai Shizenhogo Kiko*, **17**, 45–56.

Occhipinti-Ambrogi A. (2007) Global change and marine communities: alien species and climate change. *Marine Pollution Bulletin*, **55**, 342–352.

Pastorino G., Darrigran G., Martin S.M., Lunaschi L. (1993) *Limnoperna fortunei* (Dunker, 1857) (Mytilidae), nuevo bivalvo invasor en aguas del Río de la Plata. *Neotropica*, **39**, 34.

Penchaszadeh P.E., Paredes C., Salaya J.J. (2000) Reproductive cycle of the South American scallop *Amusium laurenti* (Gmelin, 1791) (Bivalvia, Pectinidae). *Aquaculture international*, **8**, 227–235.

Por F.D. (1978) *Lessepsian Migration: The Influx of Red Sea Biota into the Mediterranean by Way of the Suez Canal.* Springer, Heidelberg: 1–228.

Purchon R.D. (1968) *The Biology of the Mollusca.* Pergamon Press, Hungary.

Raj P.J.S. (2008) Oysters in a new classification of keystone species. *Resonance*, **13**, 648–654.

Rilov G., Gasith A., Benayahu Y. (2002) Effect of an exotic prey on the feeding pattern of a predatory snail. *Marine Environmental Research*, **54**, 85–98.

Sharon Y. (2006) Aspects in the ecology of two Lessepsian invasive bivalves, *Spondylus spinosus* and *Chama pacifica*, in the Eastern Mediterranean Sea. MSc Thesis, Tel Aviv University.

Spanier E., Galil B.S. (1991) Lessepsian migration – a continuous biogeographical process. *Endeavour*, **15**, 102–106.

Tillier L., Bavay A. (1905) Les mollusques testacés du Canal de Suez. *Bulletin de la Société Zoologique de France*, **30**, 170–181.

Türkmen A., Türkmen M., Tepe Y. (2005) Biomonitoring of heavy metals from İskenderun Bay using two bivalve species *Chama pacifica* Broderip, 1834 and *Ostrea stentina* Payraudeau, 1826. *Turkish Journal of Fisheries and Aquatic Sciences*, **5**, 107–111.

Vermeij G.J.l. (1996) An agenda for invasion biology. *Biological Conservation*, **78**, 3–9.

Wronski T. (2010) The molluscan bio-fouling community on the Red Sea pearl oyster beds (Mollusca: Pteriidae). *Zoology in the Middle East*, **51**, 67–73.

Yonge C.M. (1967) Form, Habit and Evolution in the Chamidae (Bivalvia) with Reference to Conditions in the Rudists (Hippuritacea). *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **252**, 49–105.

Zardi G.I., McQuaid C.D., Teske P.R., Barker N.P. (2007) Unexpected genetic structure of mussel populations in South Africa: indigenous *Perna perna* and invasive *Mytilus galloprovincialis*. *Marine Ecology Progress Series*, **337**, 135–144.

Zenetos A., Konstantinou F., Konstantinou G. (2009) Towards homogenization of the Levantine alien biota: additions to the alien molluscan fauna along the Cypriot coast. *Marine Biodiversity Records*, **2**, 156.

Zenetos A., Gofas S., Verlaque M., Cinar M.E., Raso J.E.G., Bianchi C.N., Morri C., Azzurro E., Bilecenoglu M., Froglia C., Siokou I., Violanti D., Sfriso A., San Martin G., Giangrande A., Katagan T., Ballesteros E., Ramos-Espla A., Mastrototaro F., Ocana O., Zingone A., Gambi M.C., Streftaris N. (2010) Alien species in the Mediterranean Sea by 2010. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part I. Spatial distribution. *Mediterranean Marine Science*, **11**, 381–493.

Zurel D., Benayahu Y., Or A., Kovacs A., Gophna U. (2011) Composition and dynamics of the gill microbiota of an invasive Indo-Pacific oyster in the eastern Mediterranean Sea. *Environmental Microbiology*, **13**, 1467–1476.