

Invading bivalves replaced the native Levantine populations with negligible local effects on the benthic community

Rei Diga (✉ diga.boker@gmail.com)

Ruppin Academic Center School of Marine Sciences <https://orcid.org/0000-0002-3621-6423>

Merav Gilboa

Ruppin Academic Center School of Marine Sciences

Raz Moskovich

Tel Aviv University

Neomie Darmon

Ruppin Academic Center School of Marine Sciences

Tal Amit

Tel Aviv University The George S Wise Faculty of Life Sciences

Jonathan Belmaker

Tel Aviv University The George S Wise Faculty of Life Sciences

Gitai Yahel

Ruppin Academic Center School of Marine Sciences

Research Article

Keywords: East Mediterranean, Lessepsian migration, invasive species, *Spondylus spinosus*, *Chama pacifica*, *Brachidontes pharaonis*, *Pinctada radiata*, *Malleus regula*

Posted Date: May 10th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1518803/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Constructing the Suez Canal connected the Red Sea and the Mediterranean Sea, allowing rapid marine bio-invasion. Over the last century, several bivalve species have invaded the Levantine basin, yet their distribution and impact on the benthic community have not been thoroughly studied. Large-scale benthic surveys along the rocky substrate of the Israeli Mediterranean coastline indicate that invading bivalves now dominate the rocky environment, reaching densities of tens to hundreds of individuals per m². No native bivalve specimens were found in any of the transects surveyed. The small-scale ecological effects of the established invading populations on the benthic community were examined using *in-situ* exclusion experiment where all invading bivalves were either physically removed or poisoned and kept in place to maintain the physical effect of the shells. Surprisingly, the experimental exclusion showed little measurable effect of bivalve presence on the invertebrate community in close vicinity (~1 m). Bivalves presence had a small, but statistically significant, effect only on the community composition of macroalgae, increasing the abundance of some filamentous macroalgae, and reducing the cover of turf. The generally low impact of invading bivalves could be due to (1) wave activity and local currents dispersing the bivalve excreta, (2) high grazing pressure, possibly by invading herbivorous fish, reducing the bottom-up effect of increased nutrient input by the bivalves, or (3) the natural complexity of the rocky habitat masking the contribution of the increased complexity associated with the bivalve's shell. We found that established invading bivalves have replaced native bivalve species, yet their small-scale effects on the benthic community seem to be negligible.

Introduction

Marine and coastal ecosystems worldwide are being invaded at an extraordinary rate as the result of human activities and climate change that increases opportunities for new species to be introduced and subsequently establish a population (Chan and Briski 2017). Nevertheless, only a small proportion of the studies of invasive species assess the ecological effects subsequent to the invasion, especially in coastal habitats (Watkins et al. 2021). Moreover, recent introductions are more frequently studied than long-established invading species (Strayer et al. 2006; Florencio et al. 2019). Although most studies emphasize the negative effects of biological invasions, other studies indicate that non-native species have no or even positive effects on their new environment (Ruesink et al. 2005; Schlaepfer et al. 2011; Florencio et al. 2019).

The class Bivalvia (~ 8,000 marine species) includes the economically important mussels, oysters, scallops, and clams. The vast majority of bivalves are suspension feeders - they use their modified gills to remove suspended particles from water that they pump through their mantle cavity (Bracken 2004; Dame 2012; Gosling 2015; Vaughn and Hoellein 2018). The water is then excreted with metabolic waste that contains, among other metabolites, ammonium, dissolved organic matter, feces, and pseudo-feces (the latter is excreted through the inhalant opening). Through their feeding and excretion activity bivalves import (and recycle) large quantities of organic particulate matter from the water column into the benthic ecosystem (Bracken 2004; Dame 2012; Gosling 2015; Vaughn and Hoellein 2018).

Bivalve invasion is a widespread phenomenon that often results in dramatic impacts on local ecosystems due to the formation of high-density populations in a short period (Sousa et al. 2009; Higgins and Vander Zanden 2010; Escobar et al. 2018). The effects of invading bivalves on their new environment vary between the pelagic and the benthic zones (Sardiña et al. 2008; Sousa et al. 2009; Higgins and Vander Zanden 2010; Strayer 2010). Phytoplankton communities are directly affected by the invading bivalve grazing activity that commonly reduces phytoplanktonic biomass and change community composition (Cloern 1982; Higgins and Vander Zanden 2010; Strayer 2010). The bivalve feeding mechanism reduces suspended matter (organic and inorganic particles) and increases water clarity (Higgins and Vander Zanden 2010; Sousa et al. 2014). This results in deepening the photic zone and may enhance the growth of periphyton and macrophytes (Higgins and Vander Zanden 2010; Dame 2012; Gosling 2015). Moreover, both in mesocosms and *in-situ* experiments a profound macroalgae biomass increase was observed in the presence of bivalves (Bracken and Nielsen 2004; Bracken 2004).

The bivalve shell acts as a substrate for sessile species including algae, other bivalves, and a variety of invertebrates. It also increases the complexity and heterogeneity of the habitat by creating new microhabitats (Gutierrez et al. 2003; Sousa et al. 2009). Moreover, the bivalve shell can serve as a refuge for small organisms, including young stages of economically important species, from predation and abiotic stress (Fernandez et al. 1993; Gutierrez et al. 2003; Sousa et al. 2009). Fluid transport is also affected by the presence of bivalve shells via micro- and macroscopic changes in water flow and infiltration into the sediment (Gutierrez et al. 2003; Sousa et al. 2009). Due to their ability to control and/or modulate the availability of resources to other species (via physical and/or biological effects such as feeding and excretion of metabolic waste), bivalves are often referred to as ecosystem engineers (Jones et al. 1996; Crooks 2002).

The effects of invading bivalves on the benthic fauna are complex, and may include: (1) increase in biomass and diversity of associated fauna following the invasion (Crooks 2002; Sousa et al. 2009). In South American rivers, where the invasive golden mussel (*Limnoperna fortunei*) formed dense populations, invertebrates were 27–100% more numerous and consisted of 43–100% more biomass than in areas without the mussels (Sylvester et al. 2007). (2) Competitive exclusion of native bivalves (Safriel and Sasson-Frostig 1988; Rilov et al. 2004; Sarà et al. 2008; Strayer and Malcom 2018). (3) Top-down control on other sessile invertebrates through predation of planktonic larvae (Cowden et al. 1984; Crooks and Khim 1999; Strayer 2010). (4) Small or undetectable effects on the invertebrate community and native bivalves (Richardson 2020). More intricate effects are second-order interactions where, for example, the presence of the invasive Asian date mussel (*Musculista senhousia*) had consistent negative effects on the asexual propagation of eelgrass in California (Reusch and Williams 1998; Crooks and Khim 1999). The effects listed are not mutually exclusive and can result in fundamentally different effects of invasion.

The opening of the Suez Canal in 1869 initiated migration and transportation of Indo-Pacific species from the Red Sea into the Mediterranean Sea. This led to a rapid and unprecedented rate of marine bio-invasions, especially to the Levantine basin (South-East Mediterranean, Galil 2000, 2008; Rilov and Galil

2009). Hundreds of species - principally mollusks, fish, crustaceans, polychaeta, and macrophytes - have become established along the Levantine coasts, including 40 species of invading bivalves which have established populations (Galil 2000; Zenetos et al. 2010; Albano et al. 2021).

Information on native bivalve and benthic community composition in the subtidal of the Levantine basin is scant. Early reports show two native mytilids (*Mytilus galloprovincialis* and *Mytilaster minimus*) inhabiting the intertidal zone, and two oysters (*Chama gryphoides* and *Spondylus gaederopus*) inhabiting the subtidal zone (Lipkin and Safriel 1971; Barash and Danin 1992; Fishelson 2000; Crocetta et al. 2013). A monitoring program of the fish and benthic communities, including bivalves, was established in 2015 by the Israel Nature and Parks Authority (INPA), and reported that all the bivalves sampled during 2015 were invasive except one specimen (Frid and Yahel 2018). To our knowledge, very few studies have dealt with the distribution patterns of native bivalves (Safriel and Sasson-Frostig 1988), or their physiology (Sarà and Pusceddu 2008; Galimany et al. 2011 and reference therein) in the Mediterranean Sea. Recent studies have shown a severe decline in dozens of native invertebrate species populations in the Levantine basin (Rilov 2016), with an almost complete extirpation of native bivalve species in some habitats (Albano et al. 2021). Although it is clear that native species are less abundant than in the past, it is not clear whether invading species have outcompeted and functionally replaced them (Steger et al. 2021).

Several invading bivalves dominate local communities in the Levantine basin and may have replaced native bivalves (Zurel et al. 2012; Rilov 2013; Crocetta et al. 2013). The pearl oyster, *Pinctada radiata* (Leach, 1814; family Margaritidae) was one of the first invasive mollusks recorded in the Mediterranean (first observed in 1874) and became abundant in the Levantine basin (Galil 2008) with evidence of spreading into the western Mediterranean basin (Kersting and Hendriks 2021). The invading mussel *Brachidontes pharaonis* (P. Fischer, 1870; family Mytilidae) covers large swaths of the shallow rocky substrate (Rilov et al. 2004). These populations undergo boom and bust cycles that displace and eliminate native species from this habitat (Rilov et al. 2004; Rilov and Galil 2009). Note that the origin and status of *B. pharaonis* is currently unresolved (Belmaker et al. 2021). A new invading mytilid, identified as *Perna perna* (Linnaeus, 1758; family Mytilidae), has started to form dense populations at some localities and may enhance local whelk populations (Douek et al. 2021). In the subtidal zone, the invading oysters *Spondylus spinosus* (Schreibers, 1793; family Spondylidae) and *Chama pacifica* (Broderip, 1835; family Chamidae) are reported to account for most of the invertebrate rock cover and biomass (Fishelson 2000; Shabtay et al. 2014; Frid and Yahel 2018; Rilov et al. 2018). In some localities, aggregations of these species are modifying the three-dimensional structure of the rocky habitats by forming small oyster reefs that are new to this area (Zurel et al. 2012; Rilov 2013; Shabtay et al. 2014). However, to date, the densities, distribution patterns, and population structures of invading bivalves are not well documented along the Levantine basin (Zurel et al. 2012; Crocetta et al. 2013; Shabtay et al. 2014). Importantly, despite the magnitude of bivalve invasions in the Levantine basin, the ecological effects on the benthic community have not been thoroughly studied.

The current study examined the ecological effects of long-established invaders on the benthic community of the Levantine basin coastline, an environment exposed to intensive bio-invasions over the past 150

years (Por 1971; Galil 2008). To that end, we addressed two main questions: (1) what are the invading bivalve distribution patterns along the Israeli Mediterranean coastline? (2) How and to what extent do invading bivalves affect the macroalgae and invertebrate populations in their immediate vicinity? To answer these questions, we used large-scale SCUBA surveys along the rocky substrate of the Israeli Mediterranean coastline and conducted an *in-situ* exclusion experiment in which all bivalves were either physically removed or poisoned and kept in place to preserve the physical effect of their shells. The benthic community was monitored in 48 small (1.1 m²) experimental plots by detailed visual and photographic surveys and the algal growth rate was measured by using settlement plates over an annual cycle. Our study presents new data on the distribution of invading bivalve populations, decades after their invasion, and examines their ecological effects on a benthic community that is heavily affected by both other invaders and climate change.

Materials And Methods

Study area and benthic community description

The study was conducted on subtidal rocky outcrops (2–26 m depth) of the central and northern parts of the Israeli Mediterranean coastline (Fig. 1a). Most of the Israeli shelf is covered with loose sediment dominated by quartz sand in the nearshore (up to 50 m depth) where patches of rocky outcrops become more abundant toward the north. These rocky outcrops are composed of submerged aeolianite ridges (calcareous cemented sand dune called “Kurkar”) that run parallel to the coastline (Emery et al. 1960; Lipkin and Safriel 1971). The benthic species inhabiting subtidal hard substrates are documented in species checklists and inventories of native and invading species, but the community composition is poorly described (Fishelson 2000; Einav and Israel 2008; Israel and Einav 2017). The macroalgae community is mainly dominated by turf algae along with patches of canopy and erect macroalgae of both native and non-native species (Rilov et al. 2018). It should be noted that the term ‘turf’ suffers from inconsistent and vague definitions in the literature (Connell et al. 2014). Hereafter, we refer to ‘turf’ as a low-lying benthic algal mat shorter than two cm, which is mainly a mixture of filamentous algae (e.g., *Polysiphonia* spp.) and other heavily grazed algae.

Underwater visual surveys

To determine the contribution of invading bivalves to the local benthic community, and to study their distribution along the Mediterranean Israeli coastline, we conducted large-scale underwater surveys within the framework of the Israel Nature and Parks Authority’s (INPA) marine reserves monitor program “Marine Bioblitz”. The surveys were conducted at four sites: Achziv, Shikmona, Dor-Habonim, and Gdor (hereafter refer to as 'sites', Fig. 1a) during the spring and fall of 2019. To ensure good representation of the rocky outcrops in each site, sampling points (4–13 per site) were predetermined on a bathymetric map and then marked with surface buoys from a small skiff. SCUBA divers used the marks as a starting point and laid longshore line transects along the rock contour. Sampling depth was adapted to the distribution of rocky outcrops in each site and ranged between 2–26 m. Due to the scale of the survey, we

trained several survey teams. Before the onset of the surveys, teams practiced and perform cross-calibrations trials between surveyors to validate the reproducibility of the method.

Line transects (n = 263 transects for bivalve density, of which 196 also recorded percent cover of bivalves and other invertebrates) were 10 m long and 2–6 transects were surveyed at each predetermined sampling point. A fit of the line to the local relief (to represent different rocky features such as overhangs, crevices, etc.) was achieved by using several small fisher weights attached to the lines with plastic clips. Lines were surveyed at 0.1 m intervals (100 points per line). At every point along the line, we documented the substrate type (rock, sediment covering the rock, or algae) or invertebrate present to allow percent cover estimation. If an invertebrate was observed, the maximum length was measured using a plastic caliper to allow subsequent estimation of density and size-frequency distributions (Zvuloni and Belmaker 2016). Bivalves were identified to species level (when possible), but other invertebrates (> 1 cm) were classified to a higher taxonomic level (phylum or class) to ensure consistency among samplers (see table S1 in Online Resource 1). In cases where the bivalve shell was closed, it was impossible to discriminate between *Spondylus spinosus* and *Chama pacifica*. Since all examined specimens (60) were tentatively identified as *S. spinosus*, we designated all surveyed 'rocky oysters' as *S. spinosus*. However, we note that this classification may also contain *C. pacifica* specimens. Efforts are now being made to further clarify the taxonomic affiliation of these 'rocky oysters'.

Bivalve exclusion experiment

To assess the effects of invading bivalves on the local macroalgae and invertebrate communities an *in-situ* experiment with three treatments was deployed at the Gdor site (32°24'07.2"N; 34°51'30.3"E). Treatment plots consisted of 0.3 x 0.3 m quadrats with a 0.4 m width buffer zone surrounding each plot to eliminate the effects of bivalves near the main quadrat. The total treated area of each plot and buffer zone that surrounded it was 1.1 m². The treatments included: (1) 'bivalve removal'- where all bivalves within a plot boundary and a surrounding buffer zone were physically removed. This treatment removed both the biological and physical effects of bivalves. (2) 'Bivalve poisoning'-where all bivalves within a plot and surrounding buffer zone were poisoned, retaining their empty shells in place, and thus removing only the biological effects of the bivalve activity. (3) 'Control plot' - marked identically to the treatment plots but received no treatment (Fig. 1b).

To increase the statistical power and minimize the confounding effect of plot location and orientation, the experiment was organized in triplets (removal, poison, and control). A total of 16 triplets (48 plots) were positioned at depths of 7–12 m. The distances between the plots within each triplet was ~ 1.5 m. Within each triplet, the plots were deployed in the same orientation, minimizing differences in the effect of light, currents, wave activity, etc. The removal and poisoning treatments were applied simultaneously (maximum of two days apart) in each triplet. The number of bivalves removed or poisoned, as well as their taxonomic affiliation and status (dead or alive) was documented for each plot. Bivalves were physically removed using a hammer and chisel. Poisoning of bivalves was done using 8% formaldehyde injections. For *Spondylus spinosus*, a four mm small hole was drilled (NEMO hammer drill, NEMO® power

tools) in the shell as close as possible to the pallial line, and five mL of formaldehyde was injected into the organism tissue after which the hole was immediately sealed with a small amount of epoxy putty (Aquamend®) to prevent leakage into the environment. A small amount of the epoxy putty was also applied to the anterior of the shell to keep both valves attached after the bivalve death. For the smaller and less armored *Pinctada radiata* and *Malleus regula*, two mL of formaldehyde was injected after piercing the shell with a stainless-steel needle. A small amount of epoxy polymer was placed on their byssus threads to prevent detaching after the death. *Brachidontes pharaonis* was rarely (< 5) observed at the experimental plots and specimens were removed from both removal and poison plots.

Post-treatment monitoring

Algae growth rate

To monitor the effect of the presence of invading bivalves on the total macroalgae growth rate, settlement plates were placed at each plot (total of 48 plots, n = 16 triplets) for 3–5 weeks. To account for seasonal differences in the algal growth rates, this experiment was repeated four times: Spring 2020, Fall 2020, and Spring 2021 (in Spring 2020 two experiments with 8 triplets each were deployed and combined for the analysis). Plates were made of 0.12 x 0.12 m plastic squares attached to the top of an four kg diving weight. Plastic netting was positioned over the plate to prevent grazing by large herbivores (see photo S3 in Online Resource 1). On retrieval, each plate was collected into a zip-lock bag and transferred in a dark cool box to the lab where it was drained and stored at -20 °C until analysis.

To quantify the total algae growth rate on each plate, chlorophyll-*a* was used as a proxy for the photosynthetic biomass. In the lab, the bulk of the algae biomass was scraped into a glass jar using a putty knife, the remaining material was removed using high-pressure jets of NaCl solution (Waterpik, Magic-Jet®, 40 gr L⁻¹ NaCl) and collected onto a glass fiber filter, GF/D 47 mm diameter (Whatman®). The scrapings and filter were extracted together with the Hot DMSO (Dimethyl Sulfoxide) method as described in Suari et al. (2019), with some modifications. Briefly, the GF/D filter and algae scraping were transferred to 40 mL EPA vials (cat no:9-105, Thermo Fisher Scientific®), eight mL DMSO was added to each vial, and the vials were tightly closed and incubated in the dark at 60°C for 20 minutes. After cooling to room temperature, 16 mL of 90% buffer acetone was added to each vial, the vial was vigorously mixed using a vortex mixer and stored overnight in the dark at 4°C. Chlorophyll-*a* fluorescence was measured using a calibrated fluorometer (either TD-700 or Trilogy, Turner designs®) equipped with a non-acidification Chlorophyll-*a* kit (Welschmeyer 1994).

Macroalga community (photographic survey)

To measure post-treatment shifts in the macroalgae community composition and account for seasonality, five photo-quadrat surveys were conducted during 2020 and 2021. Surveys were conducted only when sea conditions were permissive, and visibility allowed high-quality photography (July 2020, August 2020, November 2020, December 2020, and May 2021). In each survey, a 0.3 x 0.3 m quadrat was placed on the central plot and a set of photos was taken using an underwater camera (tough TG-5,

Olympus®) with two adjustable lights (Sea Dragon 2500, SeaLife®). Unidentified algae were collected for identification by Dr. Alvaro Israel. Photo orientation and edges were adjusted using Faststone image viewer (Faststone soft®) and uploaded to a designated 'source' (<https://coralnet.ucsd.edu/>; source name: Algal composition Michmoret) in the CoralNet platform (Beijbom et al. 2015). A set of 100 points was randomly imposed on each photo and each point was categorized as substrate (rock and loose sediment), sessile invertebrates (phylum or class), or algae. Algae were categorized to the lowest taxonomic rank possible (mainly genus, see table S4 in Online Resource 1). Points where the photo was out of focus or too dark for identification and points that fell on the quadrat frame were excluded (median of 11 points per quadrat). Percent cover was calculated as the number of data points for each category divided by the total number of data points after excluding the unidentified points.

Invertebrate surveys

To measure post-treatment shifts in the invertebrate community composition, three surveys were performed 3, 7, and 12 months after the treatments (July 2020, November 2020, and April 2021). All invertebrates larger than 0.5 cm in the main quadrat of each plot were counted and identified to the lowest taxonomic level possible (see summary table S5 in Online Resource 1). In addition to the detailed count-based survey, the percent cover of both algae and invertebrates was concurrently documented. For this, the point intercept method (PIM) was applied on a 0.3 x 0.3 m quadrat with a 3 cm grid (49 points). Algae were classified as one of three categories: crustose coralline algae (CCA), algae shorter than 2 cm, and algae longer than 2 cm. Invertebrates (> 1 cm) were classified to the class or phylum level (see table S6 in Online Resource 1).

Data analysis

Statistical analysis was carried out within the R programming language (version 4.1.1) in the Rstudio environment (version 1.4.1717, (R Core Team 2020)). Data are presented as the mean ± 95% confidence interval for the mean, unless otherwise stated. The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Distribution patterns of the invading bivalves

Percent cover was calculated for each transect as the number of data points listed for a category divided by the total number of points identified for that transect (normally 100). Abundances and size-frequency distributions were deduced using the method of Zvuloni & Belmaker (2016) which transforms point-intercept measurements to unbiased count-based indices. Based on the size of each individual and the configuration of the sampling unit (the number of points and distance between the points) an 'effectively sampled area' (ESA, m²) is calculated according to Eq. 1 of Zvuloni & Belmaker (2016):

$$\text{Eq. 1. } ESA_i = \bigcup_{k=1}^n \pi R_{i(k)}^2$$

Where ESA_i is the effectively sampled area for organism i with a radius r_i , k is the sampling point index along the line, n is the total number of sampling points, and U is the union of the areas of all n circles with radius r_i along the transect line. ESA_i is much larger for large individuals and smaller for small individuals, thereby correcting the inherent bias of the overrepresentation of larger organisms in standard point-sampling techniques. The density of an individual i encountered during a point intercept survey (PIM) can be calculated as $1/ESA_i$. For example, a single 5 cm bivalve spotted on a 10 m line with 0.1 m intervals will have an ESA of 0.196 m² and hence a calculated density of 5.11 individuals m⁻². The total density of a certain taxon was calculated for each transect as the sum of the calculated densities of all individuals from that taxon in the transect. For example, in a transect in which we encountered three bivalves with a diameter of 2, 5, and 8 cm, their respective ESAs would be 0.031, 0.196 and, 0.5 m², and hence, their calculated densities ($1/ESA_i$) would be 32, 5.1, and 2.0 individuals m⁻², respectively, producing a total estimated density of 39.1 individuals m². The code (R script) for corrected density calculations is provided in the Online Resource 2. Once the unbiased densities of each taxon were calculated for each transect, standard diversity indices could be calculated. Size-frequency distributions were not calculated for *Brachidontes pharaonis* because the majority of individuals were smaller than the minimum size threshold (1 cm) and for *Malleus regula* that were spotted only sporadically during the survey. Examples of the sampling form and data table used for the surveys can be found in (Diga 2022).

Effects of the presence of invading bivalves on the macroalgae community

Algae growth rate

Differences in macroalgae growth rates ($\mu\text{g Chl-}a \text{ m}^{-2} \text{ day}^{-1}$) between treatments and months were tested using Friedman non-parametric repeated measure ANOVA on log-transformed data, since the macroalgae growth rate did not meet the assumptions of normality and sphericity.

Macroalga community (photographic survey)

Dissimilarities in the macroalgae community composition between treatments were calculated using log-transformed Bray-Curtis dissimilarity and visualized with nMDS ordination. Significance of the dissimilarities between treatments was tested with ANOSIM test (Clarke et al. 2014). Before analysis, rarely observed taxa (less than 10 counts in all surveys combined) were removed. To analyze the contribution of different taxa to the difference between treatments and the similarity within treatments, a SIMPER procedure was used (Clarke et al. 2014). This procedure identifies the taxa that are likely to be the major contributors to any difference between treatments.

Taxa which accounted for at least 5% of the total cover in at least one of the months and the total algae cover were examined more closely using a Linear Mixed Effect (LME) models with the lme4 package (Bates et al. 2014) and tested for significance with lmerTest package in R. The response was logit-transformed percent cover, and the predictor was *treatment*. To account for values of zero and one in the data the smallest cover measurable (1%) was added to all values smaller than one or subtracted from

values equal to one prior to logit transformation. *Plot* was used as a random effect (to account for the repeated measure nature of the design):

$$\text{Eq. 2. } P. \text{ cover} \sim \text{Treatment} + (1|\text{Plot})$$

For taxa that were present in all months (turf and total algae), the *month* was added as an additional random effect in Eq. 2. *Jania* spp. was present only in three months, so *month* was added as a categorical fixed effect in Eq. 2.

Effects of the presence of invading bivalves on the invertebrate community

Invertebrates α -diversity indices (percent cover, richness, effective number of species, and the total number of individuals) were calculated for each plot and survey. Each parameter was fitted with General Linear Models (GLMs) using the *glmer* function in the lme4 package in R. The predictors were *treatment* and *month* while *plot* was used as a random effect in all models (to account for the repeated measure nature of the design):

$$\text{Eq. 3. } \text{Index} \sim \text{Treatment} + \text{Month} + (1|\text{Plot})$$

A Poisson link function was used for count-based parameters (e.g., richness and number of individuals). For diversity and percent cover (logit transformed) the distribution family was Gaussian.

Dissimilarities in the invertebrate community composition between treatments and months were calculated using log-transformed Bray-Curtis and visualized with nMDS ordinations. The significance of treatments and months was tested with ANOSIM.

Disturbed areas (i.e., the treatment plots) tend to have greater variability than that of undisturbed areas (i.e., the control plots). A multivariate dispersion index (MVDISP, Warwick and Clarke 1993) was calculated (on log-transformed Bray-Curtis matrix) to compare the variability of the invertebrate community composition between the treatment in every survey. The average dispersion was calculated for each treatment (group) and tested for significance between treatments and among months with GLM as described in Eq. 3 with a Gaussian distribution.

Results

Distribution patterns

The Israeli rocky subtidal zone was dominated by benthic algae throughout the year with percent covers reaching ~ 80%. Invertebrates accounted for $8 \pm 1\%$ of the coverage (mean \pm 95% CI) and sponges, bryozoan, and invading bivalves contributed the most to the invertebrate cover (Fig. 2a). Invading bivalves were a prominent group that reached up to half of the invertebrate cover (Fig. 2a). No native bivalves were found in any of the 263 transects surveyed (total of over 2400 m).

Fig. 2. **(a)** Contribution of taxonomic groups to the overall percent cover of invertebrates (bars at the right) and the percent cover of bivalve species (bars at the left) along the Israeli rocky subtidal zone calculated over all sites and depths during two seasons in 2019 ($n = 196$, 10 m transects). Error bars represent 95% confidence intervals of the overall mean percent cover. **(b)** Mean densities ($\pm 95\%$ CI) and, **(c)** mean shell length ($\pm 95\%$ CI) of invading bivalve species. No native bivalves were observed throughout the survey. $n = 159$ and 104 , 10 m transects in spring and fall, respectively. Note that *S. spinosus* is a tentative identification and may contain specimens of *C. pacifica* (see methods for details).

The mean abundance of *S. spinosus* was 13 ± 6 individuals m^{-2} ($\pm 95\%$ CI) and 9 ± 4 individuals m^{-2} in spring and fall respectively, while *B. pharaonis* was observed in much higher densities during the fall (149 ± 91 individuals m^{-2}) in comparison to the spring (75 ± 49 individuals m^{-2}). *M. regula* was observed only during fall (Fig. 2b). The mean shell length of *S. spinosus* was 4.9 cm (Fig. 2c) and bivalves larger than 4 cm accounted for $\sim 10\%$ of the population (Fig. 3a). Small bivalves with a shell length of ~ 1 cm dominated the population during spring, whereas the size distribution of the bivalves surveyed in the fall was shifted to the right (Kruskal Wallis test, $p = 0.75$). The mean shell length of *P. radiata* was 1.8 ± 0.05 cm (Fig. 2c) and bivalves > 2 cm accounted for $\sim 30\%$ of the population (Fig. 3b). As for *S. spinosus*, the size-frequency distribution of *P. radiata* also shifted to the right in the fall surveys (Kruskal Wallis test, $p = 0.22$).

Fig. 3. Size frequency distributions of **(a)** *S. spinosus* and **(b)** *P. radiata* in Spring and Fall 2019. Unbiased frequencies were calculated using the effectively sampled area of each size category (Zvuloni & Belmaker 2016). Note the different scales on the x-axis between a and b. For *S. spinosus* $n = 78$ and 84 observations for spring and fall, respectively. For *P. radiata* $n = 31$ and 12 observations for spring and fall, respectively.

Effects of invading bivalve presence on the macroalgae community

Algae growth rate

The total algal growth rate on the plates ranged from 30 ± 5 to 140 ± 50 μg Chl-*a* $m^{-2} day^{-1}$ (see table S2 in Online Resource 1). Growth rates did not differ significantly between the treatments (see S7 in Online Resource 1) except for the poisoned treatment in spring 2020, which was significantly lower than both the removal and the control treatments (Friedman test, $p = 0.04$).

Macroalgae community composition

Percent cover of turf algae was 3–13% and 4–17% higher in the removal and poison treatments, respectively, compared to the control (depending on the month of survey) and this difference was highly significant (Fig. 4, GLM test, $p < 0.001$, table S10 in Online Resource 1). An inverse pattern was observed for *Cladophora* spp. that covered significantly less area in both poison and removal treatments during July 2020 (Fig. 4, GLM test, $p < 0.05$, table S10 in Online Resource 1). Poison and removal treatments

showed similar results suggesting that the difference from the control is due to the biological activity of the bivalves and not the physical presence of the shells.

A total of 14 genera of macroalgae were observed during the 5 photographic surveys along with turf algae which were the most ubiquitous group in the plots (Fig. 5). Multivariate analysis of the algae community composition identified a small but statistically significant difference between the treatments and control (ANOSIM, $R = 0.011$, $p < 0.05$, see nMDS ordinations S8 in Online Resource 1). The overall dissimilarity (calculated by SIMPER procedure) between the poison and removal treatments to the control was small (26 and 27%, respectively, table S9 in Online Resource 1). Six taxa accounted for more than 95% of the dissimilarities, with turf algae contributing most of the dissimilarity between treatments.

Effects of invading bivalves on the invertebrate community

A total of 46 taxa were observed during the three surveys (July 2020, November 2020, and April 2021). Treatment had no effects on most α diversity indices (Fig. 6 and GLM table S11 in Online Resource 1). The total number of individuals was significantly lower in the removal treatment compared to the control (Fig. 6). Significant seasonal changes were observed in both the number of individuals and percent cover of all invertebrates combined (table S11 in Online Resource 1). Multivariate analysis of the invertebrate community composition (using Bray-Curtis similarity index on log-transformed data) did not identify significant differences between the treatments and control (ANOSIM, $R = 0.0148$ $p = 0.071$, see nMDS ordinations S12 in Online Resource 1). Multivariate dispersion index (MVDISP, Warwick and Clarke 1993) of the invertebrate community structure was higher in the removal treatment (0.52 ± 0.02) in comparison to the poison treatment (0.46 ± 0.03) and control (0.46 ± 0.02), indicating a more variable invertebrate assemblage.

Discussion

In this study we evaluated the current status of bivalves inhabiting rocky outcrops along the Israeli Mediterranean coastline and assessed their ecological effects on the nearby local benthic community. By using a large-scale SCUBA survey, we found that invading bivalves have become prominent on the rocky subtidal outcrops along the Israeli coastline. No native bivalve specimens were encountered during the study. Despite the high density of invading bivalves and their prominence along the rocky outcrops, our *in-situ* experiment showed no effect of exclusion of invading bivalves on the invertebrate community or the overall macroalgae growth rate in their immediate vicinity. Bivalve exclusion did show a small but statistically significant effect on the community composition of the macroalgae. The results of this study suggest that the long-established populations of invading bivalves have negligible ecological impacts on the benthic community in their immediate vicinity.

Replacement of native bivalves

The record of native bivalves inhabiting rocky habitats in the Israeli coastline and other areas in the Levantine basin is largely based on presence-absence data (Fishelson 2000, Crocetta et al. 2013).

Reconstruction of the historical bivalve richness was recently made from death assemblages (Albano et al. 2021) but for most species, other than *Brachidontes pharaonis* and *Mytilus galloprovincialis* (Chintiroglou et al. 2004; Sará et al. 2008; Sarà et al. 2018, and references therein), no information is available regarding their ecological effects on the surrounding benthic communities. The last reports in the Israeli coastline of native species such as *Mytilus galloprovincialis* and *Mytilaster minimus* are from 2000 (Fishelson 2000) while *Chama gryphoides* was observed once in 2015 (R. Diga and R. Yahel, unpublished data) and recently in low numbers in northern Israel (see supplementary in Albano et al. 2021). Although Israel is within the distribution area of *Spondylus gaederopus*, only one live specimen was reported to date (Barash & Danin 1992). Burrowing bivalves such as *Lithophaga* are common (Rilov et al. 2018, Albano et al. 2021) but due to their cryptic nature, were outside the scope of this study.

The establishment of invading bivalve populations from the Indo-Pacific area in the Levantine basin started around 150 years ago and well established populations of invading bivalves along the Israeli coastline have been reported for at least four decades (Mienis et al. 1993a, b; Galil 2008). Our results suggest that invading bivalves are currently a prominent group of the invertebrate community on the rocky subtidal outcrops of the Israeli coastline. No native bivalve specimen was encountered in over > 150 dives conducted in this study. A similar absence of native species was reported by experts from the Steinhardt Museum of Natural History in similar surveys carried out between 2015–2017 as part of the INPA marine reserves monitoring program (R. Diga and R. Yahel, unpublished data). In some locations, invading bivalves accounted for half of the invertebrates cover and reached densities ranging from tens to hundreds of individuals m^{-2} for *S. spinosus* and *B. pharaonis*, respectively (Fig. 2). These findings corroborate previous reports from the Israeli coastline (Zurel et al. 2012; Shabtay et al. 2014; Rilov et al. 2018). An absence of native bivalves on rocky substrates was also reported for the Lebanese coastline (Crocetta et al. 2013), suggesting that the native bivalve fauna that inhabits the rocky substrate has been replaced by invading bivalves throughout the Levantine basin. It is still not clear whether invading bivalves outcompete native bivalves (Safriel and Sasson-Frostig 1988) or whether the disappearance of the native populations was driven by the dramatic change of conditions in the Levantine basin such as rising sea temperature, elevated salinity, and oligotrophication (Givan et al. 2018; Galil et al. 2021; Steger et al. 2021).

A previous study on the reproduction cycle of *S. spinosus* from this area suggested a spawning period during the summer months (Shabtay et al. 2015) while several species in the genus *Spondylus* were reported to settle 2–3 months after fertilization (Loor et al. 2016). The dominance of small individuals during the spring (Fig. 3, left-side) may imply that the recruitment time of *S. spinosus* occurs between late fall and early winter. The shift to larger individuals during fall for both *S. spinosus* and *P. radiata* (Fig. 3, right-side), may reflect an enhanced growth period between spring and fall when seawater temperature increases (Ozer et al. 2022) or mortality of small recruits.

Lack of small-scale effects

There are numerous examples of dramatic changes following the establishment of invading bivalves but most of these examples are in soft-bottom environments (Higgins and Vander Zanden 2010; Strayer and Malcom 2018). In contrast, our exclusion experiment over hard substrate showed no measurable effect of bivalve presence on the invertebrate community in their immediate vicinity and only a small effect on the community composition of the macroalgae (Figs. 5 and 6). This result was similar for both poisoning and removal treatments suggesting that the physical effect of the shell presence is small. Considering the high density and prominence of the invading bivalve populations on the rocky outcrops, the lack of strong effects is surprising, especially considering the intense biological activity of these populations. For example, Amit et al. (unpublished data) estimated a filtration rate of several cubic meters of seawater per m^2 per day¹ and excretion rate of feces and pseudo-feces of tens of mg of organic matter per m^2 per day¹ for the *Spondylus spinosus* population alone.

Several environmental factors may mask the bivalves' physical and biological effects on the benthic community. The excreta of the invading bivalves (feces, pseudo-feces, and dissolved organic and inorganic matter) may be quickly dispersed by local currents and wave activity. Under low energy conditions, a concentration boundary layer depleted from phytoplankton and enriched with excreta is often formed above dense populations of suspension-feeding bivalves. In contrast, under high energy conditions (waves or currents) this boundary layer is quickly dissipated (Fr chet te et al. 1989; Wildish and Kristmanson 1997; Ackerman and Loewen 2001). Similarly, waves and currents may also quickly disperse feces and pseudo-feces (Wotton and Malmqvist 2001). The Israeli coastline is impacted by relatively high waves and the annual closure depth (where wave activity affects sediment transport) is between 4.7 to 9.1 m (Bitan and Zviely 2020). Hence, the experimental plots positioned between 7–12 m were exposed to strong wave action and the resulting currents throughout the year. In this relatively high energy environment, bivalve excreta are likely to be quickly dispersed to other areas (including adjacent treated plots), thereby masking the bivalve effects.

The decrease of turf algae in the presence of invading bivalves and the increase in some seasonal algae (such as *Cladophora* spp.) relative to the poison and removal treatments suggests that some algae may benefit from the biological activity of invading bivalves under natural conditions and in the presence of grazers. The similar growth rates between treatments and control where grazing was prevented (by using fish excluders over the settlement plates) can be attributed to the small size of the plates that allowed only the growth of early stages of development and succession of the algae. The fish excluders hindered the growth of high-canopy and erect macroalgae that were present in the experimental plots under natural conditions.

Seasonal algae are fast-growing with high demands for nutrients (N and P, Pedersen and Borum 1996). Coupling between bivalves and specific macroalgae was observed both in mesocosms and *in-situ* experiments (Bracken and Nielsen 2004; Bracken 2004) with large macroalgae biomass increases when bivalves were present. Surprisingly, the effects we measured were small (< 20%, Fig. 4), suggesting that other factors, potentially the high grazing pressure induced by herbivorous fish, such as *Siganus rivulatus* and *S. luridus*, may set a strong top-down control on the macroalgae, masking the effects of the bivalve

excreta. Since their introduction in the middle of the 20th century, *S. rivulatus* and *S. luridus* have exerted heavy grazing pressure on the macroalgae community along the Mediterranean Israeli coastline (Yeruham et al. 2020). These rabbitfishes comprise up to 90% of the herbivorous fish and one-third of the total fish biomass in rocky habitats along the Israeli coastline (Lazarus et al. 2020 unpublished data), and exert a continuous and relentless grazing pressure on the benthic algae community (Sala et al. 2011; Pickholtz et al. 2018; Yeruham et al. 2020). Sala (2011) has shown that in rocky reefs in Turkey, these species can turn algal meadows to turf barrens. Despite the high abundance of grazers and subsequently the dominance of turf in the Levantine basin, the presence of invading bivalves may have beneficial effects to specific macroalgae which may increase the total algal biomass, diversity, and functionality.

The physical contribution of the bivalve shells was small. We found a slight, but statistically significant, increase of the total number of invertebrate individuals in the poison treatments compared to control, a pattern absent in the removal treatment (Fig. 6). In addition, we found higher MVDISP index in the removal treatment compared to the poison and control. Since there was no specific pattern of species that settled in each of the treatment areas, these results may simply reflect settling of invertebrates in the newly opened space of removal plots. In soft bottom sediments, invading bivalves increase complexity by stabilizing the sediment and form 3D structures (Gutierrez et al. 2003). However, on the structurally complex rocky outcrops, the bivalve shells seem to have little effect on the invertebrate community. Bivalve shells and the addition of new hard substrate by secondary settlement creates complex features that can serve as a potential niche for other benthic organisms and result in substantial changes of the benthic environment and the benthic communities (Gutierrez et al. 2003; Sousa et al. 2009). In comparison to soft-bottom habitats, the rocky subtidal outcrops of the Israeli coastline have a much higher level of complexity and include features such as overhangs, cracks, crevices, and vertical surfaces. In fact, the invading bivalves studied appear to be more abundant in these natural features which may supply protection from wave activity and abrasion by loose sediment. Therefore, the physical contribution of the bivalve shells in these relatively complex environments may be less important, and the shell is less likely to serve as a new substrate for other sessile invertebrates. In rocky environments, the new substrate formed by invading bivalve shells seems to have little effect on the invertebrate community since species that attach to bivalve shells can also attach to surrounding rocks.

The current study adds new insights on the status of bivalves along the East Mediterranean coastline and especially on the ecological effects of long-established invading populations. We found that invading bivalves have replaced native bivalves that inhabits rocky substrates, yet they have no measurable effects on the nearby invertebrate community and only a small effect on the macroalgae community. However, our experimental design was limited in scope and does not allow informed predictions of the rate and nature of the benthic community shifts that may follow large-scale and long-term bivalve exclusion. Moreover, we did not survey the nocturnal benthic motile community, which includes detritivores (such as, hermit crabs and sea-cucumbers) that may benefit from the bivalve excretion of feces and pseudo-feces (Stewart and Haynes 1994; Ricciardi et al. 1997). Information on the ecological effects of native bivalves (e.g., *Mytilus galloprovincialis*) in the Mediterranean sea is scant (Bateman and Bishop 2017) and suggest that the presence of bivalves can increase the abundance of specific groups

of invertebrate such as polychaeta and crustacean (see Table 3 in Chintiroglou et al. 2004; Çinar et al. 2008). Since no information is available on the density of *M. galloprovincialis* in the past along the Israeli coastline, or on the distribution and ecological effects of other native species, we do not know how bivalves impacted their surroundings in the past, nor whether the now established invading populations has modified the ecological function of the rocky ecosystem of Levantine basin relative to pre-invasion conditions.

The unprecedented rate and magnitude of bio-invasions in marine coastal areas emphasizes the need to understand how and to what extent invading species impact their new environment. Most studies focus on the occurrence of new invading species but much less effort has been devoted to the study of ecological effects subsequent to the invasion (Watkins et al. 2021), especially through manipulative experiments (Katsanevakis et al. 2014). It should be noted that the effects of invading species that established populations long ago, can change dramatically with time (Strayer et al. 2006). Our study examined the ecological effects of an invading bivalve community decades after its establishment and showed negligible small-scale (~ 1 m) effects on the nearby benthic community. While our results do not preclude potential large-scale (~ 100s m) effects, they stress the need for long-term ecological assessment of the role of invading species in the local ecosystem.

Declarations

Funding

This work was funded by a grant from anonymous philanthropic fund to the INPA, ISF grant 249/21, and BSF Grant 2012089 to GY.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation and data collection were performed by Rei Diga, Merav Gilboa, Raz Moskovich, Neomie Darmon, and Tal Amit. Analysis was performed by Rei Diga, Jonathan Belmaker, and Gitai Yahel. The first draft of the manuscript was written by Rei Diga, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Ackerman JD, Loewen MR (2001) Benthic-pelagic coupling over a zebra mussel reef in western Lake Erie. *Limnology and*
2. Albano PG, Steger J, Bošnjak M et al (2021) Native biodiversity collapse in the eastern Mediterranean. *Proceedings B* 288:20202469. <https://doi.org/10.1098/rspb.2020.2469>
3. Barash A, Danin Z (1992) Annotated List of Mediterranean Molluscs of Israel and Sinai. The Israel Academy of Sciences and Humanities, Jerusalem
4. Bateman DC, Bishop MJ (2017) The environmental context and traits of habitat-forming bivalves influence the magnitude of their ecosystem engineering. *Mar Ecol Prog Ser* 563:95–110. <https://doi.org/10.3354/meps11959>
5. Bates D, Mächler M, Bolker B, Walker S (2014) Fitting linear mixed-effects models using lme4. *J Stat Softw* arXiv. <https://doi.org/10.18637/jss.v067.i01>. :1406
6. Beijbom O, Edmunds PJ, Roelfsema C et al (2015) Towards automated annotation of benthic survey images: variability of human experts and operational modes of automation. *PLoS ONE* 10:e0130312. <https://doi.org/10.1371/journal.pone.0130312>
7. Belmaker J, Abelson A, Haddas-Sasson M et al (2021) Potential Pitfalls in the Definition of Lessepsian Migrants: The Case of *Brachidontes*. In: Jawad LA (ed) *The Arabian Seas: Biodiversity, Environmental Challenges and Conservation Measures*. Springer International Publishing, Cham, pp 1293–1307
8. Bitan M, Zviely D (2020) Sand Beach Nourishment: Experience from the Mediterranean Coast of Israel. *J Mar Sci Eng* 8:273. <https://doi.org/10.3390/jmse8040273>
9. Bracken MES (2004) Invertebrate-mediated nutrient loading increases growth of an intertidal macroalga. *J Phycol* 40:1032–1041. <https://doi.org/10.1111/j.1529-8817.2004.03106.x>
10. Bracken MES, Nielsen KJ (2004) Diversity of intertidal macroalgae increases with nitrogen loading by invertebrates. *Ecology* 85:2828–2836. <https://doi.org/10.1890/03-0651>
11. Chan FT, Briski E (2017) An overview of recent research in marine biological invasions. *Mar Biol* 164:121. <https://doi.org/10.1007/s00227-017-3155-4>
12. Chintiroglou C-C, Damianidis P, Antoniadou C et al (2004) Macrofauna biodiversity of mussel bed assemblages in Thermaikos Gulf (northern Aegean Sea). *Helgol Mar Res* 58:62–70. <https://doi.org/10.1007/s10152-003-0169-8>
13. Çınar ME, Katağan T, Koçak F et al (2008) Faunal assemblages of the mussel *Mytilus galloprovincialis* in and around Alsancak Harbour (Izmir Bay, eastern Mediterranean) with special emphasis on alien species. *J Mar Syst* 71:1–17. <https://doi.org/10.1016/j.jmarsys.2007.05.004>
14. Clarke KR, Gorley RN, Somerfield PJ, Warwick RM (2014) *Change in marine communities: An approach to statistical analysis and interpretation*, 3rd edition. Primer-E Ltd, Plymouth
15. Cloern JE (1982) Does the benthos control phytoplankton biomass in south San Francisco Bay. *Mar Ecol Prog Ser* 9:191–202

16. Connell SD, Foster MS, Airoidi L (2014) What are algal turfs? Towards a better description of turfs. *Mar Ecol Prog Ser* 495:299–307. <https://doi.org/10.3354/meps10513>
17. Cowden C, Young CM, Chia FS (1984) Differential predation on marine invertebrate larvae by two benthic predators. *Mar Ecol Prog Ser* 14:145–149
18. Crocetta F, Bitar G, Zibrowius H, Oliverio M (2013) Biogeographical homogeneity in the eastern Mediterranean Sea. II. Temporal variation in Lebanese bivalve biota. *Aquat Biology* 19:75–84. <https://doi.org/10.3354/ab00521>
19. Crooks JA (2002) Characterizing ecosystem-level consequences of biological invasions: The role of ecosystem engineers. *Oikos* 97:153–166
20. Crooks JA, Khim HS (1999) Architectural vs. biological effects of a habitat-altering, exotic mussel, *Musculista senhousia*. *J Exp Mar Biol Ecol* 240:53–75. [https://doi.org/10.1016/s0022-0981\(99\)00041-6](https://doi.org/10.1016/s0022-0981(99)00041-6)
21. Dame RF (2012) *Ecology of Marine Bivalves: An Ecosystem Approach*, 2nd edition. CRC Press, Boca Raton
22. Diga R (2022) Small-scale ecological effects of invading bivalves in the Levantine basin. Ruppiner Academic Center
23. Douek J, Paz G, Gayer K et al (2021) An outbreak of *Perna perna* (Linnaeus, 1758) (Mollusca, Bivalvia, Mytilidae) in the Eastern Mediterranean. *Biol Invasions Records* 10:136–148. <https://doi.org/10.3391/bir.2021.10.1.15>
24. Einav R, Israel A (2008) Checklist of seaweeds from the Israeli Mediterranean: Taxonomical and ecological approaches. *Isr J Plant Sci* 56:127–191. <https://doi.org/10.1560/ijps.57.1-2.127>
25. Emery KO, Neev D, Bentor YK (1960) *Mediterranean Beaches of Israel*. Geological Survey Of Israel, Jerusalem
26. Escobar LE, Mallez S, McCartney M et al (2018) Aquatic invasive species in the Great Lakes region: an overview. *Reviews in Fisheries Science & Aquaculture* 26:121–138. <https://doi.org/10.1080/23308249.2017.1363715>
27. Fernandez M, Iribarne O, Armstrong D (1993) Habitat selection by young-of-the-year Dungeness crab *Cancer magister* and predation risk in intertidal habitats. *Mar Ecol Prog Ser* 92:171–171
28. Fishelson L (2000) Marine animal assemblages along the littoral of the Israeli Mediterranean seashore: the Red-Mediterranean Seas communities of species. *Italian J Zool* 67:393–415. <https://doi.org/10.1080/11250000009356345>
29. Florencio M, Lobo JM, Bini LM (2019) Biases in global effects of exotic species on local invertebrates: a systematic review. *Biol Invasions* 21:3043–3061. <https://doi.org/10.1007/s10530-019-02062-1>
30. Fréchette M, Butman CA, Geyer WR (1989) The importance of boundary-layer flows in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L: Food supply to mussels. *Limnol Oceanogr* 34:19–36. <https://doi.org/10.4319/lo.1989.34.1.0019>

31. Frid O, Yahel R (2018) Marine BioBlitz- Biological survey of the Israelis MPAs in the Mediterranean Sea- Spring and Fall 2015. Israel Nature and Parks Authority
32. Galil BS (2008) Alien species in the Mediterranean Sea—which, when, where. why? *Hydrobiologia* 606:105–116. <https://doi.org/10.1007/s10750-008-9342-z>
33. Galil BS (2000) A sea under siege – alien species in the Mediterranean. *Biol Invasions* 2:177–186. <https://doi.org/10.1023/A:101005701047>
34. Galil BS, Mienis HK, Hoffman R, Goren M (2021) Non-indigenous species along the Israeli Mediterranean coast: tally, policy, outlook. *Hydrobiologia* 848:2011–2029. <https://doi.org/10.1007/s10750-020-04420-w>
35. Galimany E, Ramón M, Ibarrola I (2011) Feeding behavior of the mussel *Mytilus galloprovincialis* (L.) in a Mediterranean estuary: A field study. *Aquaculture* 314:236–243. <https://doi.org/10.1016/j.aquaculture.2011.01.035>
36. Givan O, Edelist D, Sonin O, Belmaker J (2018) Thermal affinity as the dominant factor changing Mediterranean fish abundances. *Glob Change Biol* 24:e80–e89. <https://doi.org/10.1111/gcb.13835>
37. Gosling E (2015) *Marine Bivalve Molluscs*, 2nd edition. John Wiley & Sons, Chichester
38. Gutierrez JL, Jones CG, Strayer DL, Iribarne OO (2003) Mollusks as ecosystem engineers: The role of shell production in aquatic habitats. *Oikos* 101:79–90
39. Higgins SN, Vander Zanden MJ (2010) What a difference a species makes: a meta-analysis of dreissenid mussel impacts on freshwater ecosystems. *Ecol Monogr* 80:179–196
40. Israel A, Einav R (2017) Alien seaweeds from the Levant basin (Eastern Mediterranean Sea), with emphasis to the Israeli shores. *Isr J Plant Sci* 64:99–110. <https://doi.org/10.1080/07929978.2016.1257091>
41. Jones CG, Lawton JH, Shachak M (1996) Organisms as ecosystem engineers. In: Samson FB, Knopf FL (eds) *Ecosystem Management: Selected Readings*. Springer, New York, NY, pp 130–147
42. Katsanevakis S, Wallentinus I, Zenetos A et al (2014) Impacts of invasive alien marine species on ecosystem services and biodiversity: a pan-European review. *Aquat Invasions* 9:391–423. <https://doi.org/10.3391/ai.2014.9.4.01>
43. Kersting DK, Hendriks IE (2021) On the lookout for the endangered, only to find the invasive. *Front Ecol Environ* 19:442–442. <https://doi.org/10.1002/fee.2413>
44. Lazarus M, Frid O, Yahel R (2020) Marine BioBlitz- Biological survey of the Israelis MPAs in the Mediterranean sea- summary report for years 2015, 2017, 2019. Israel Nature and Parks Authority
45. Lipkin Y, Safriel U (1971) Intertidal zonation on rocky shores at Mikhmoret (Mediterranean, Israel). *J Ecol* 59:1–30. <https://doi.org/10.2307/2258448>
46. Loor A, Ortega D, Lodeiros C, Sonnenholzner S (2016) Early life cycle and effects of microalgal diets on larval development of the spiny rock-scallop, *Spondylus limbatus* (Sowerby II, 1847). *Aquaculture* 450:328–334. <https://doi.org/10.1016/j.aquaculture.2015.08.012>

47. Mienis HK, Galili E, Rapoport J (1993a) On the presence of the Indo-Pacific bivalve *Chama pacifica* in the Eastern Mediterranean. *Gloria Maris* 32:13–18
48. Mienis HK, Galili E, Rapoport J (1993b) The spiny oyster, *Spondylus spinosus*, a well-established Indo-Pacific bivalve in the Eastern Mediterranean off Israel (Mollusca, Bivalvia, Spondylidae). *Zool Middle East* 9:83–92. <https://doi.org/10.1080/09397140.1993.10637650>
49. Ozer T, Gertman I, Gildor H, Herut B (2022) Thermohaline Temporal Variability of the SE Mediterranean Coastal Waters (Israel) – Long-Term Trends, Seasonality, and Connectivity. *Front Mar Sci* 8. <https://doi.org/10.3389/fmars.2021.799457>
50. Pedersen MF, Borum J (1996) Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. *Mar Ecol Prog Ser* 142:261–272
51. Pickholtz RSM, Kiflawi M, Friedlander AM, Belmaker J (2018) Habitat utilization by an invasive herbivorous fish (*Siganus rivulatus*) in its native and invaded range. *Biol Invasions* 20:3499–3512. <https://doi.org/10.1007/s10530-018-1790-4>
52. Por FD (1971) One hundred years of Suez canal-A century of lessepsian migration: Retrospect and viewpoints. *Syst Zool* 20:138. <https://doi.org/10.2307/2412054>
53. R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Version 4.1.1 URL <https://www.R-project.org/>
54. Reusch TBH, Williams SL (1998) Variable responses of native eelgrass *Zostera marina* to a non-indigenous bivalve *Musculista senhousia*. *Oecologia* 113:428–441. <https://doi.org/10.1007/s004420050395>
55. Ricciardi A, Whoriskey FG, Rasmussen JB (1997) The role of the zebra mussel (*Dreissena polymorpha*) in structuring macroinvertebrate communities on hard substrata. *Can J Fish Aquat Sci* 54:2596–2608. <https://doi.org/10.1139/f97-174>
56. Richardson TD (2020) The ecological consequences of nonindigenous *Corbicula fluminea* establishment on a benthic macroinvertebrate community. *Aquat Invasions* 15:382–407. <https://doi.org/10.3391/ai.2020.15.3.03>
57. Rilov G (2016) Multi-species collapses at the warm edge of a warming sea. *Sci Rep* 6:36897. <https://doi.org/10.1038/srep36897>
58. Rilov G (2013) Regional extinctions and invaders' domination: an ecosystem phase-shift of Levant reefs. *Rapport de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée* 40:782–783
59. Rilov G, Benayahu Y, Gasith A (2004) Prolonged lag in population outbreak of an invasive mussel: a shifting-habitat model. *Biol Invasions* 6:347–364
60. Rilov G, Galil B (2009) Marine bioinvasions in the Mediterranean Sea – history, distribution and ecology. In: Rilov G, Crooks JA (eds) *Biological Invasions in Marine Ecosystems: Ecological, Management, and Geographic Perspectives*. Springer, Berlin, Heidelberg, pp 549–575

61. Rilov G, Peleg O, Yeruham E et al (2018) Alien turf: overfishing, overgrazing and invader domination in south-eastern Levant reef ecosystems. *Aquat Conserv* 28:351–369.
<https://doi.org/10.1002/aqc.2862>
62. Ruesink JL, Lenihan HS, Trimble AC et al (2005) Introduction of non-native oysters: ecosystem effects and restoration implications. *Annu Rev Ecol Evol Syst* 36:643–689.
<https://doi.org/10.1146/annurev.ecolsys.36.102003.152638>
63. Safriel UN, Sasson-Frostig Z (1988) Can colonizing mussel outcompete indigenous mussel? *J Exp Mar Biol Ecol* 117:211–226. [https://doi.org/10.1016/0022-0981\(88\)90058-5](https://doi.org/10.1016/0022-0981(88)90058-5)
64. Sala E, Kizilkaya Z, Yildirim D, Ballesteros E (2011) Alien marine fishes deplete algal biomass in the Eastern Mediterranean. *PLoS ONE* 6:e17356. <https://doi.org/10.1371/journal.pone.0017356>
65. Sarà G, Porporato EMD, Mangano MC, Mieszkowska N (2018) Multiple stressors facilitate the spread of a non-indigenous bivalve in the Mediterranean Sea. *J Biogeogr* 45:1090–1103.
<https://doi.org/10.1111/jbi.13184>
66. Sarà G, Pusceddu A (2008) Scope for growth of *Mytilus galloprovincialis* (Lmk., 1819) in oligotrophic coastal waters (Southern Tyrrhenian Sea, Italy). *Mar Biol* 156:117–126.
<https://doi.org/10.1007/s00227-008-1069-x>
67. Sarà G, Romano C, Mazzola A (2008) A new lessepsian species in the western Mediterranean (*Brachidontes pharaonis* Bivalvia: Mytilidae): density, resource allocation and biomass. *Mar Biodivers Rec* 1:955. <https://doi.org/10.1017/S175526720600087X>
68. Sarà G, Romano C, Widdows J, Staff FJ (2008) Effect of salinity and temperature on feeding physiology and scope for growth of an invasive species (*Brachidontes pharaonis* - MOLLUSCA: BIVALVIA) within the Mediterranean sea. *J Exp Mar Biol Ecol* 363:130–136.
<https://doi.org/10.1016/j.jembe.2008.06.030>
69. Sardiña P, Cataldo DH, Boltovskoy D (2008) The effects of the invasive mussel, *Limnoperna fortunei*, on associated fauna in South American freshwaters: importance of physical structure and food supply. *Fundamental and Applied Limnology / Archiv fur Hydrobiologie* 173:135–144.
<https://doi.org/10.1127/1863-9135/2008/0173-0135>
70. Schlaepfer MA, Sax DF, Olden JD (2011) The potential conservation value of non-native species. *Conserv Biol* 25:428–437. <https://doi.org/10.1111/j.1523-1739.2010.01646.x>
71. Shabtay A, Rilov G, Benayahu Y (2015) The Indo-Pacific oyster *Spondylus spinosus* Schreibers, 1793 in the Eastern Mediterranean Sea: reproductive features. *Molluscan Res* 35:206–212.
<https://doi.org/10.1080/13235818.2015.1007534>
72. Shabtay A, Tikochinski Y, Benayahu Y, Rilov G (2014) Preliminary data on the genetic structure of a highly successful invading population of oyster suggesting its establishment dynamics in the Levant. *Mar Biol Res* 10:407–415. <https://doi.org/10.1080/17451000.2013.814790>
73. Sousa R, Gutiérrez JL, Aldridge DC (2009) Non-indigenous invasive bivalves as ecosystem engineers. *Biol Invasions* 11:2367–2385. <https://doi.org/10.1007/s10530-009-9422-7>

74. Sousa R, Novais A, Costa R, Strayer DL (2014) Invasive bivalves in fresh waters: impacts from individuals to ecosystems and possible control strategies. *Hydrobiologia* 735:233–251. <https://doi.org/10.1007/s10750-012-1409-1>
75. Steger J, Bošnjak M, Belmaker J et al (2021) Non-indigenous molluscs in the Eastern Mediterranean have distinct traits and cannot replace historic ecosystem functioning. *Glob Ecol Biogeogr*. <https://doi.org/10.1111/geb.13415>
76. Stewart TW, Haynes JM (1994) Benthic Macroinvertebrate Communities of Southwestern Lake Ontario Following Invasion of *Dreissena*. *J Great Lakes Res* 20:479–493. [https://doi.org/10.1016/S0380-1330\(94\)71164-3](https://doi.org/10.1016/S0380-1330(94)71164-3)
77. Strayer DL (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshw Biol* 55:152–174. <https://doi.org/10.1111/j.1365-2427.2009.02380.x>
78. Strayer DL, Eviner VT, Jeschke JM, Pace ML (2006) Understanding the long-term effects of species invasions. *Trends Ecol Evol* 21:645–651. <https://doi.org/10.1016/j.tree.2006.07.007>
79. Strayer DL, Malcom HM (2018) Long-term responses of native bivalves (Unionidae and Sphaeriidae) to a *Dreissena* invasion. *Freshw Sci* 37:697–711. <https://doi.org/10.1086/700571>
80. Suari Y, Dadon-Pilosof A, Sade T et al (2019) A long term physical and biogeochemical database of a hyper-eutrophicated Mediterranean micro-estuary. *Data in Brief* 27:104809. <https://doi.org/10.1016/j.dib.2019.104809>
81. Sylvester F, Boltovskoy D, Cataldo D (2007) The invasive bivalve *Limnoperna fortunei* enhances benthic invertebrate densities in South American floodplain rivers. *Hydrobiologia* 589:15–27. <https://doi.org/10.1007/s10750-007-0708-4>
82. Vaughn CC, Hoellein TJ (2018) Bivalve impacts in freshwater and marine ecosystems. *Annu Rev Ecol Evol Syst* 49:183–208. <https://doi.org/10.1146/annurev-ecolsys-110617-062703>
83. Warwick RM, Clarke KR (1993) Increased variability as a symptom of stress in marine communities. *J Exp Mar Biol Ecol* 172:215–226
84. Watkins HV, Yan HF, Dunic JC, Côté IM (2021) Research biases create overrepresented “poster children” of marine invasion ecology. *Conserv Lett* 14. <https://doi.org/10.1111/conl.12802>
85. Welschmeyer NA (1994) Fluorometric of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnol Oceanogr* 39:1985–1992
86. Wildish D, Kristmanson D (1997) *Benthic Suspension Feeders and Flow*. Cambridge University Press
87. Wotton RS, Malmqvist B (2001) Feces in Aquatic Ecosystems. *Bioscience* 51:537–544. [https://doi.org/10.1641/0006-3568\(2001\)051\[0537:FIAE\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2001)051[0537:FIAE]2.0.CO;2)
88. Yeruham E, Shpigel M, Abelson A, Rilov G (2020) Ocean warming and tropical invaders erode the performance of a key herbivore. *Ecology* 101:e02925. <https://doi.org/10.1002/ecy.2925>
89. Zenetos A, Gofas S, Verlaque M et al (2010) Alien species in the Mediterranean Sea by 2010. A contribution to the application of European Union’s Marine Strategy Framework Directive (MSFD).

90. Zurel D, Gophna U, Benayahu Y (2012) 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. Marine Ecology 33:261–271. <https://doi.org/10.1111/j.1439-0485.2011.00490.x>
91. Zvuloni A, Belmaker J (2016) Estimating ecological count-based measures from the point-intercept method. Mar Ecol Prog Ser 556:123–130. <https://doi.org/10.3354/meps11853>

Figures

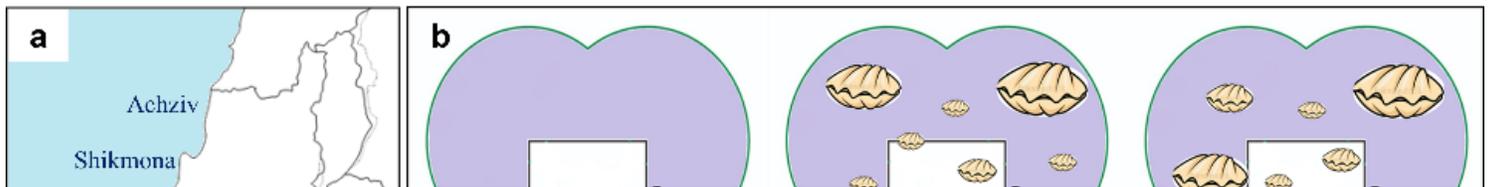
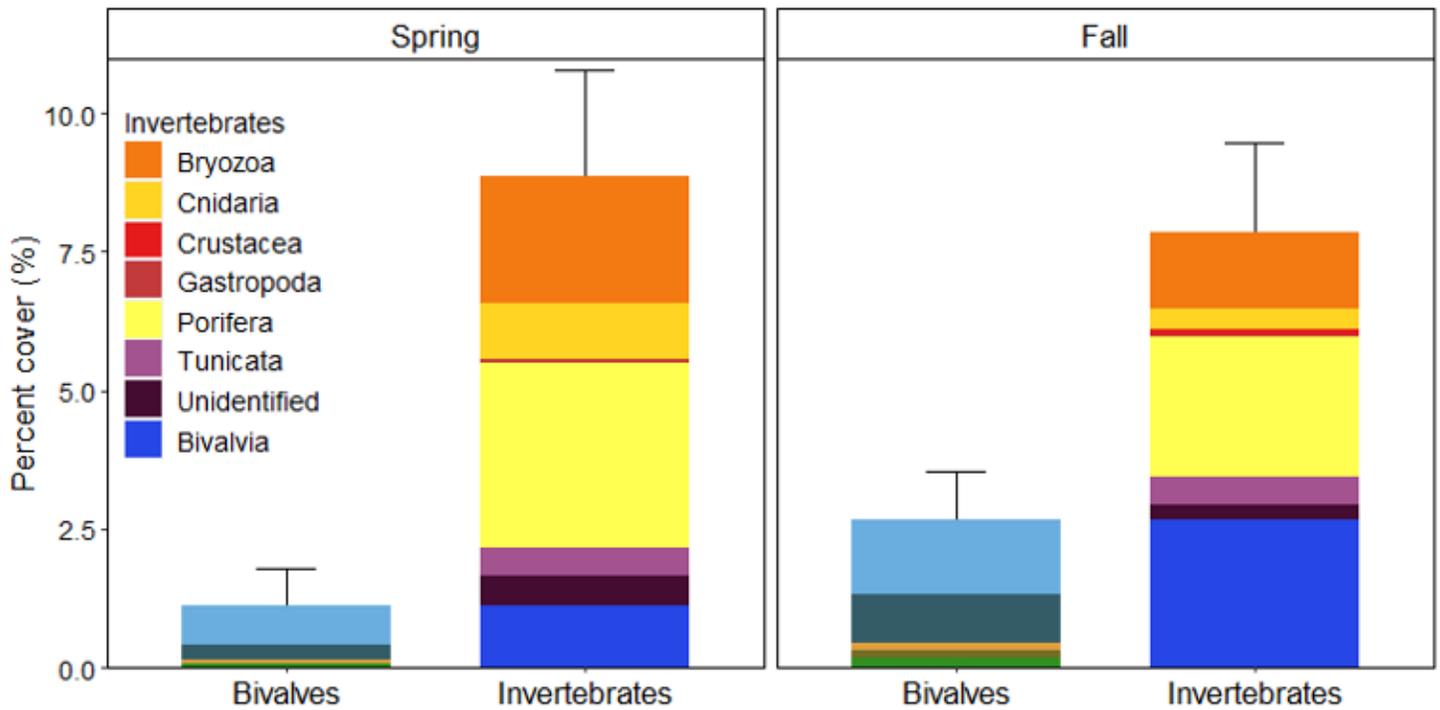


Figure 1

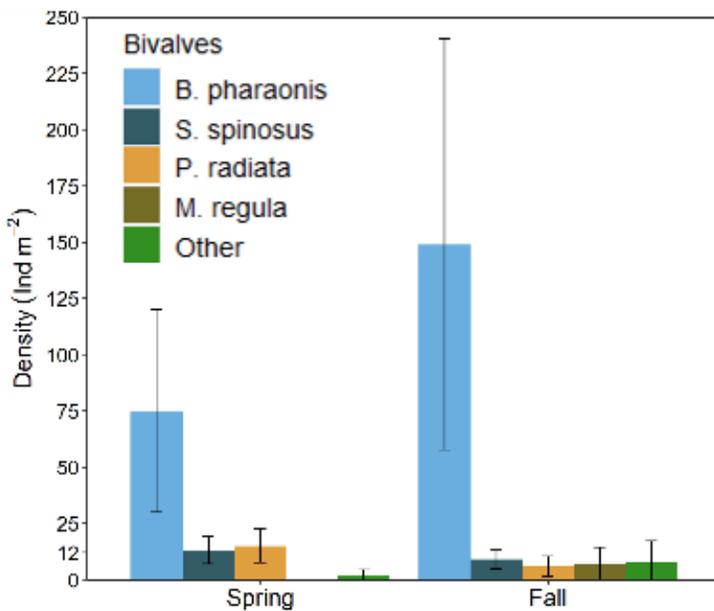
(a) Map of the Levantine basin at the south-east of the Mediterranean Sea and the four sampling sites along the Israeli coastline surveyed during spring and fall 2019. **(b)** Illustration of the experimental design with the three treatments: (1) bivalve removal- where all bivalves were physically removed, (2) bivalve poisoning- where all bivalves were poisoned, retaining their empty shells in place, (3) control plot - marked plots with no other interference. The white area represents the main quadrat which received most of the sampling effort. Light purple represents the surrounding treated buffer zone. **(c)** Examples of

experimental plots. Left - four pegs define the main quadrat boundaries, and two tags are attached in diagonal pegs for identification (see detailed protocol in Diga 2022). Right - a control quadrat with invading bivalves taken in December 2020 when turf and coralline algae (mainly *Ellisolandia* spp.) dominate the rocky subtidal. White arrows indicate the shell openings of the invading bivalve *S. spinosus*.

a. Percent cover



b. Density



c. Shell length

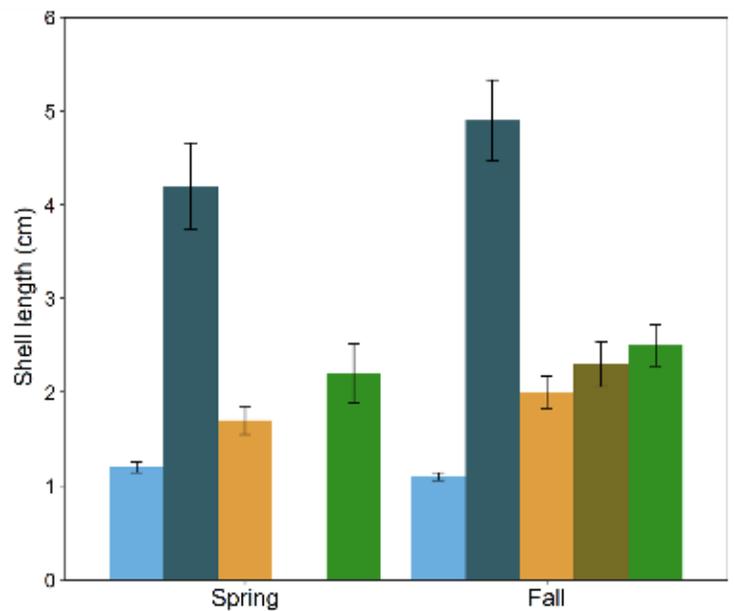
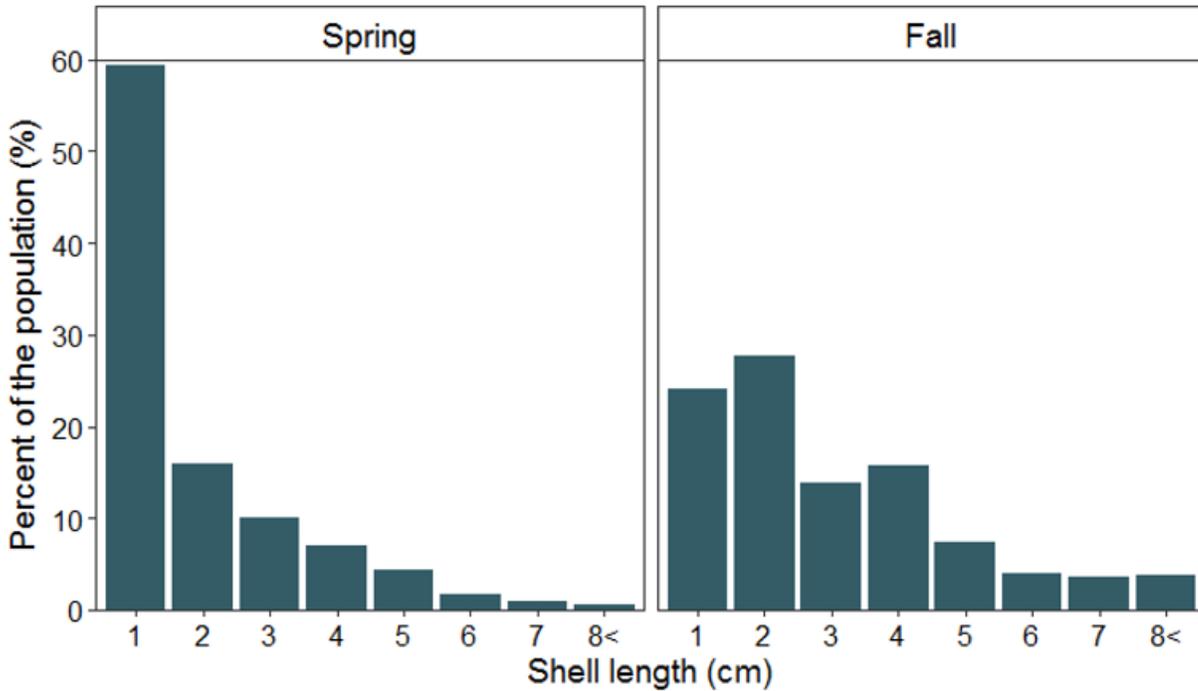


Figure 2

(a) Contribution of taxonomic groups to the overall percent cover of invertebrates (bars at the right) and the percent cover of bivalve species (bars at the left) along the Israeli rocky subtidal zone calculated over all sites and depths during two seasons in 2019 (n = 196, 10 m transects). Error bars represent 95% confidence intervals of the overall mean percent cover. **(b)** Mean densities (\pm 95% CI) and, **(c)** mean shell length (\pm 95% CI) of invading bivalve species. No native bivalves were observed throughout the survey. n=159 and 104, 10 m transects in spring and fall, respectively. Note that *S. spinosus* is a tentative identification and may contain specimens of *C. pacifica* (see methods for details).

a. *Spondylus spinosus*



b. *Pinctada radiata*

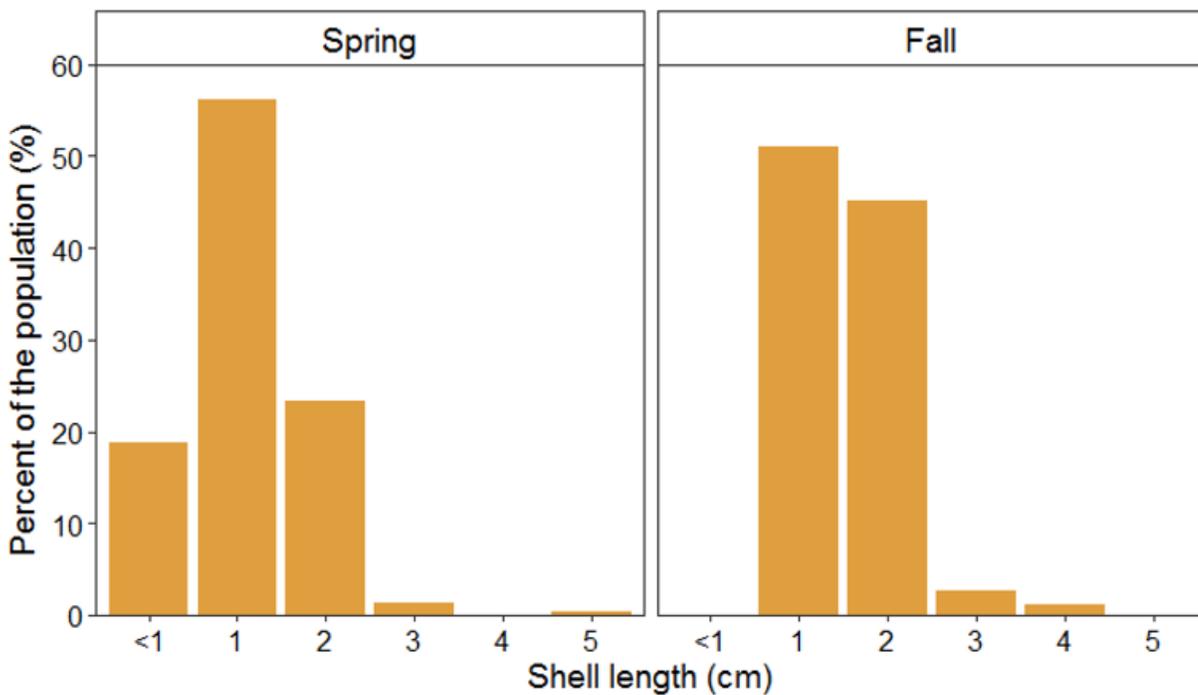


Figure 3

Size frequency distributions of (a) *S. spinosus* and (b) *P. radiata* in Spring and Fall 2019. Unbiased frequencies were calculated using the effectively sampled area of each size category (Zvuloni & Belmaker 2016). Note the different scales on the x-axis between a and b. For *S. spinosus* n=78 and 84 observations for spring and fall, respectively. For *P. radiata* n = 31 and 12 observations for spring and fall, respectively.

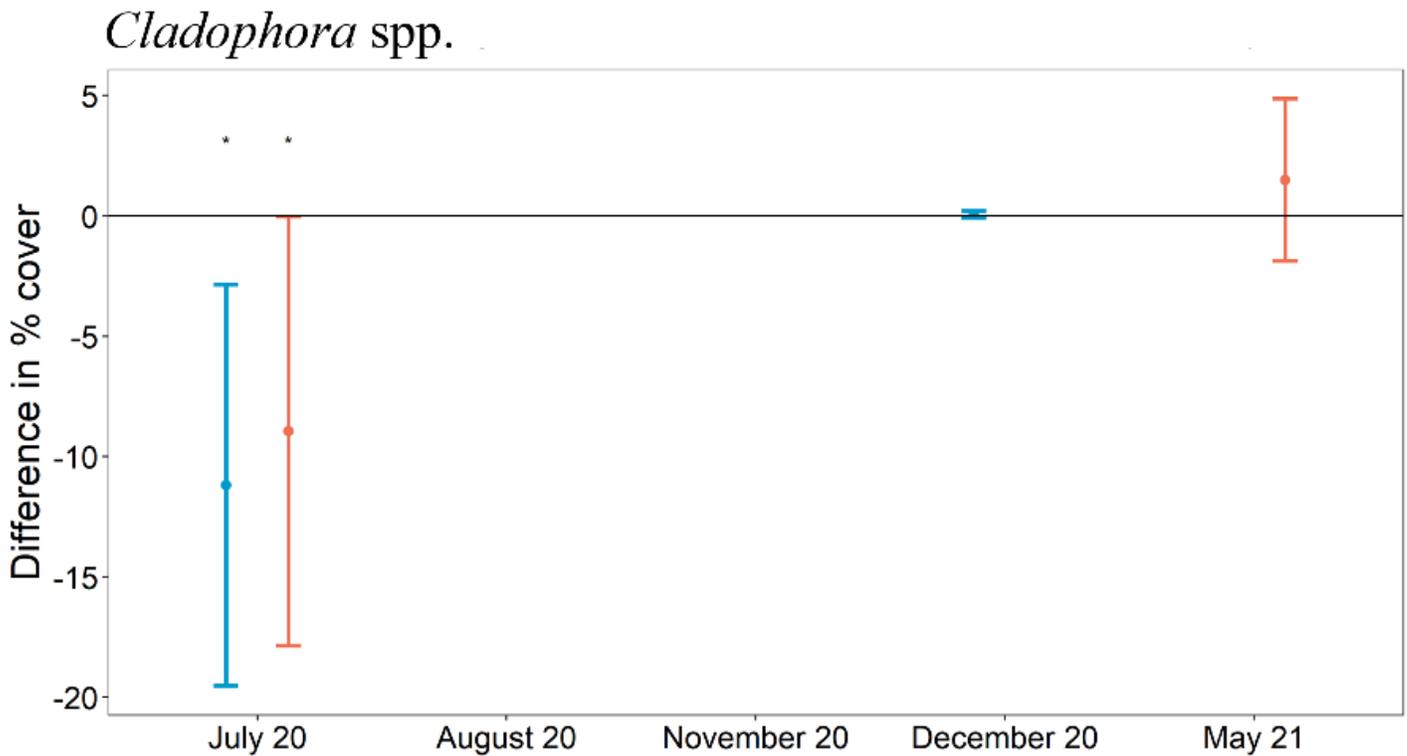
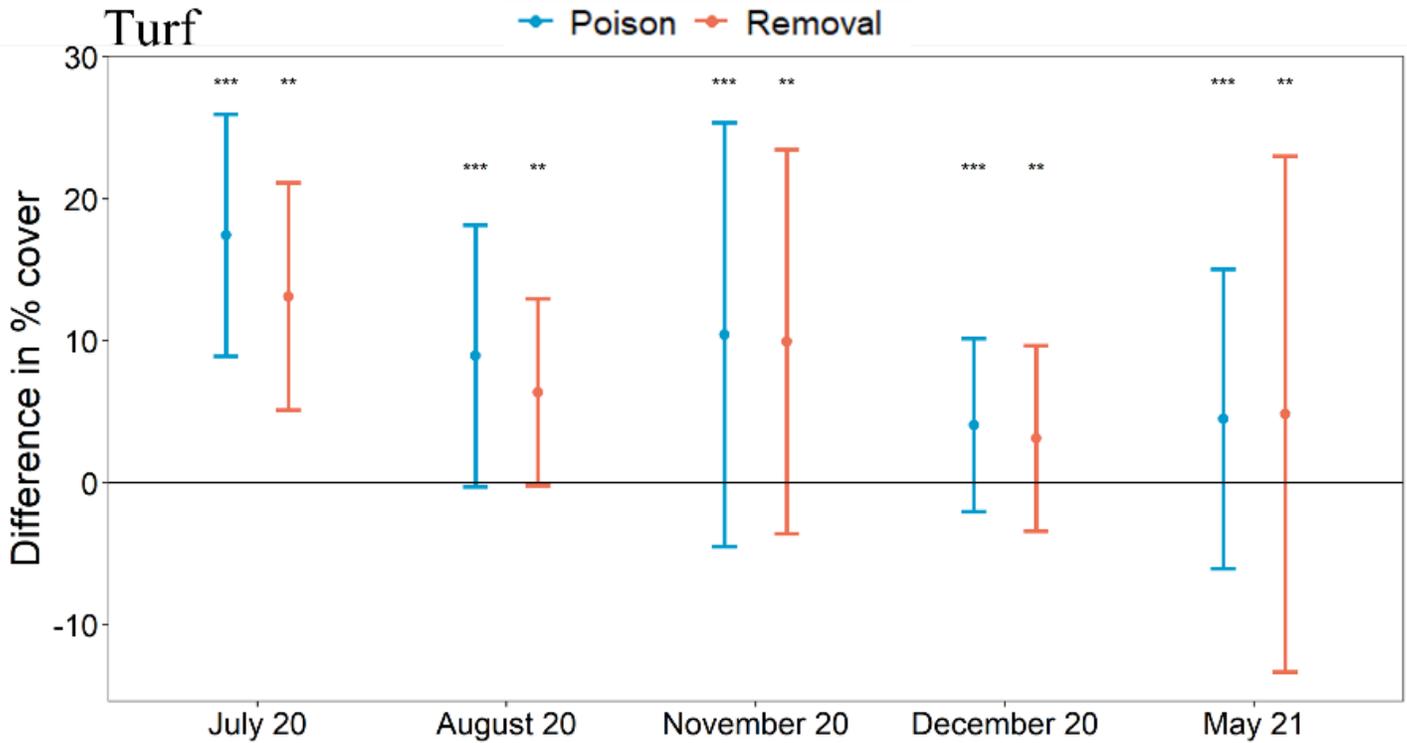


Figure 4

Differences in percent cover of turf and the macroalgae *Cladophora* spp. between treatments and control along the succession in the plots (months of survey). The differences between poison (blue) or removal (red) treatments from the control are shown. Note that *Cladophora* spp. was absent during some months.

The black horizontal line represents the expectation for no treatment effects. Data are mean \pm 95% CI over all five surveys. n=16, 16, 16, 15 and 6 triplets photographed in each month. Significance is represented by: *** p < 0.001, ** p < 0.01, * p < 0.05 based on GLM test (see table S10 in Online Resource 1).

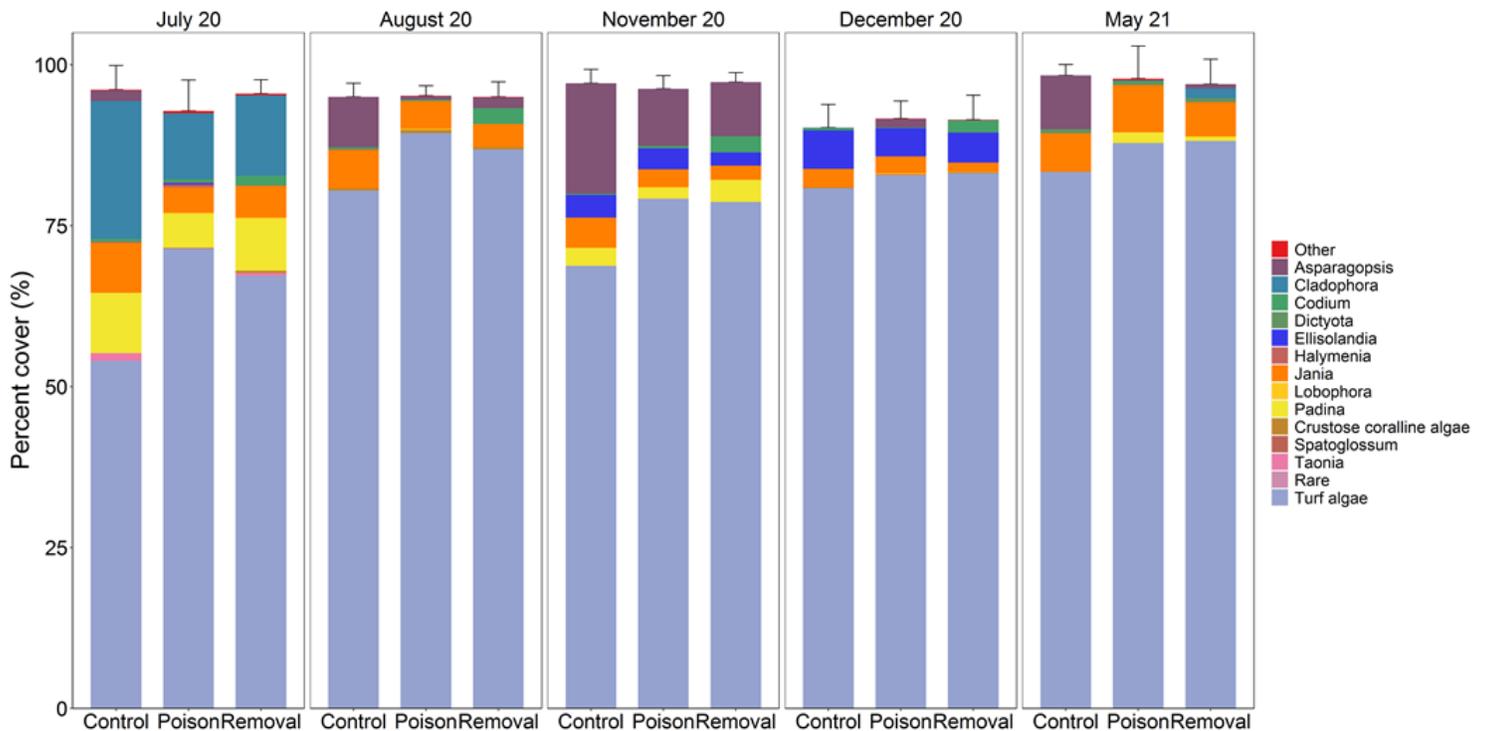


Figure 5

Mean percent cover of macroalgae in three treatments, with month of survey in the upper x-axis. Colors represent the macroalgae taxa observed. Error bars are 95% CI of the total algae cover. n=16, 16, 16, 15 and 6 triplets photographed in each month.

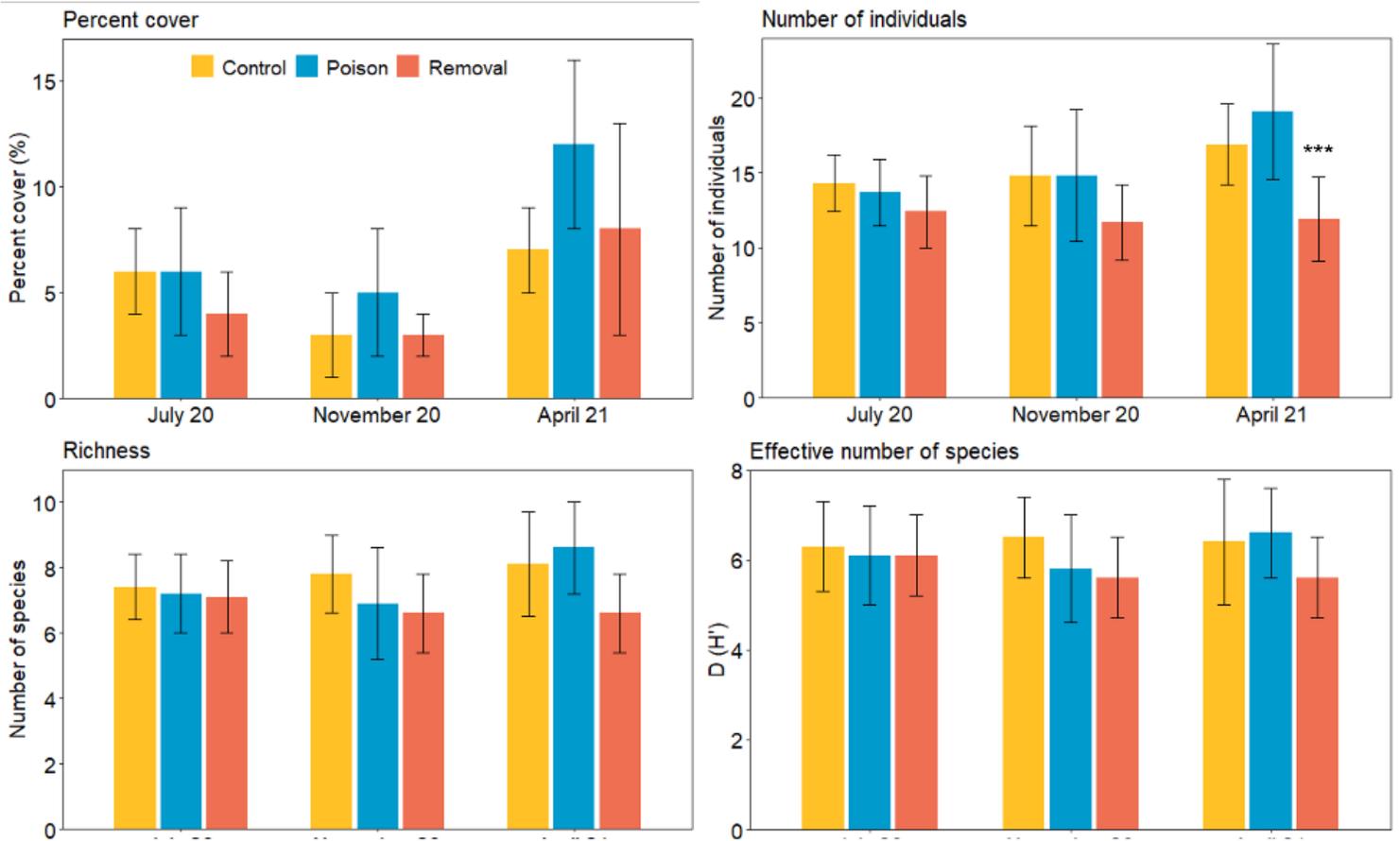


Figure 6

α diversity of the invertebrate community in the experimental plots. Mean percent cover, the total number of individuals, richness, and Shannon-Wiener diversity (effective number of species transformation, $D(H')$) during three surveys (July 2020, November 2020, and April 2021). Note the different y-axes. Colors represent the treatments: control (yellow), poison (blue), and removal (red). Error bars represent 95% CI. $n=16$ triplets in each survey (in April 2021 one quadrat of the percent cover in the poison treatment was not surveyed). No statistical significance was observed except for the number of individuals in the removal treatment (GLM test, see table S11 in Online Resource 1).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [ESM1.docx](#)
- [ESM2.r](#)