



The Symbiotic Association of Starfish *Asterias rubens* and Copepod *Scottomyzon gibberum*

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Received: 06/08/2020

Accepted: 10/08/2020

Published: 20/10/2020

Abstract

In this paper we study the specifics of the population of copepods *Scottomyzon gibberum* Scott dwell in starfish *Asterias rubens* L. in Kandalaksha Bay of the White Sea near the N.A.Pertsov White Sea Biological Station. We singled out the "average" most populated starfish size class. The distribution of copepods on the surface and the proportion of individuals in the galls depend on the type of the biotope in which populations of starfish live and salinity of the water. In the area of Polovie islands and "Babie Sea" population of starfish is higher as well as the number of cells in their coelomic fluid. The area of the carapace of copepods in the researched water area varies.

Keywords: *Asterias rubens*, *Scottomyzon gibberum*, population indices, coelomocytes, White Sea, an area of the carapace

1 Introduction

Symbiosis is widely common in nature, and symbiotic associations often play a key role in maintaining normal functioning of terrestrial, freshwater, and marine ecosystems (Smurov, 2003). To understand the nature of symbiotic associations, interactions in the system "symbiont-host", their place and role in the existence and dynamics of communities it is necessary to study various aspects, including topical and trophic interactions, the seasonal and spatial dynamics, the influence of biotope conditions and host defense mechanisms (Ieshko, Bugmyrin, & Pavlov, 2009). The object of the study is the symbiocenosis of mass littoral species of starfish *Asterias rubens* L. and copepods *Scottomyzon gibberum* Scott dwelling in them. The area of the studied species is distributed in the territory of the northern seas (Vevers, 1949). Adult females *S. gibberum* live on the surface of the starfish *A. rubens* and induce the formation of galls. Larval stage, males and young females are free-living planktonic organisms (Rettger, 1969). *S. gibberum* in the White Sea is first described by AV Smurov (Smurov, 1993). Copepods of the northern seas typically have 1.5-year life cycle with the death of females in October and November (Rettger, 1969). *S. gibberum* on the surface of starfish lead to the formation of galls due to the growth of soft host covers, in which one or more females are present (Rettger, 1969).

A. rubens is characterized by a cyclical population of copepods during the vegetative period, increasing from June to October, and during this period the proportion of males on the surface of starfish increases (A.A. Poromov&Smurov, 2014). Copepods *S. gibberum* are sensitive to low salinity, that is why migration of starfishes to the freshened areas lead to decrease in their population (Poromov & Smurov, 2014). We studied the features of the population of copepods *S. gibberum* living on the surface of starfishes *A. rubens*, the character of distribution of the copepods, described the population of different size

classes of starfishes, and the properties of the location of copepods on the body surface of *A. rubens*. The data of the population supplemented with the research results of starfish coelomic liquid cells and dimensional characteristics of copepods.

2 Materials and methods

Starfishes were collected in plastic bags near NA Pertsov White Sea Biological Station (Murmansk region, Russia (66.55359 °N, 33.10403 °E). The stations are presented in Table 1. Each starfish was placed into an individual pack. The sample size was 1015 specimens. We selected intact five-pointed starfishes from eight stations during the whole vegetation period of 2012-2013, with the average frequency of once every three weeks and 30-40 specimens for a time. At the depth of up to two meters the starfishes were collected by a net, at the depth of 3-8 meters - using diving equipment. Salinity and water temperature were measured. We measured dimensional parameters of the starfishes: Hr (the distance from madreporic plate to the inner circle, contrradius), r (the distance from the center to the inner circle, a small radius), R (the distance from the center to the great circle, a large radius). Copepods *S. gibberum* were collected from the surface of the starfish by a microscopic needle with binocular glasses with 1.8-fold increase. Female copepods were placed in 50 mm-diameter Petri dishes and fixed with 5% formalin solution for one day. After fixing females *S. gibberum* were viewed through a binocular microscope, we measured the length and the width of the carapace, and calculated the area, according to the formula for the area of an ellipse. To characterize the population of copepods on the surface of the starfish the main indicators of the number of symbionts were calculated: extensiveness, intensity, variation index, the character of distribution of the copepods on the surface of starfishes was determined as one of the proxy indicators of population status

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(Rózsa, Reiczigel, & Majoros, 2000). Coelomic fluid was extracted from the cavity of not fewer than 10 starfishes from

each station by a 1 ml syringe fitted with a 21 mm needle. The average volume of the collected liquid was 0.6 ml.

Table 1: Collection stations of starfish *Asterias rubens*

№	Collection station	Coordinates	Type of bottom communities	Type of biotope
1	The "Babie Sea"	N66.602 E33.195	<i>Mytilus edulis</i> and <i>Fucus vesiculosus</i> on rocky ground	Rocky muddy ground
2	Polovie islands	N65.573 E33.003	<i>Mytilus edulis</i> and <i>Fucus vesiculosus</i> on rocky ground	Rocky muddy ground
3	Black gap-Ermolinskaya bay	N66.332 E033.025	<i>Fucus vesiculosus</i> on rocky ground	Sandy ground with eelgrass
4	MSU WSBS pier	N66.331 E33.060	<i>Zoostera marina</i> , <i>Arenicola marina</i> on sandy ground	Sandy ground with eelgrass
5	Eremeevskie islands	N66.325 E33.071	<i>Laminaria saccharina</i> on sandy muddy ground	Rocky ground with thickets of laminaria
6	Kislaya bay	N66.321 E33.052	<i>Zoster marina</i> , <i>Arenicola marina</i> on sandy ground	Sandy ground with eelgrass
7	Chernaya river	N66.311 E32.580	<i>Mytilus edulis</i> on sandy ground in the estuary	Sandy ground with eelgrass
8	Krestovie islands	N66.520 E33.198	<i>Laminaria saccharina</i> on sandy muddy ground	Rocky ground with thickets of laminaria

The number of cells in the coelomic fluid was counted in Hemocytometer in duplication using Romanowsky-Giemsa staining solution (Venier, Maron, & Canova, 1997). The statistical analysis was done using the software IBM SPSS Statistics 20, Quantitative Parasitology 3.0. The level of significance was set as less than 0.05.

3 Results

3.1 Population of starfishes by size classes

The analysis of size structure of starfishes was conducted using principal component analysis, which allowed to convert three measured parameters into one. This conversion makes it possible to evaluate the connection between the three-dimensional parameters altogether and the population of copepods. The regression analysis of the integral size indicator of the first component (which determines the age variability - an increase in size with age) was further conducted and the number of copepods was estimated.

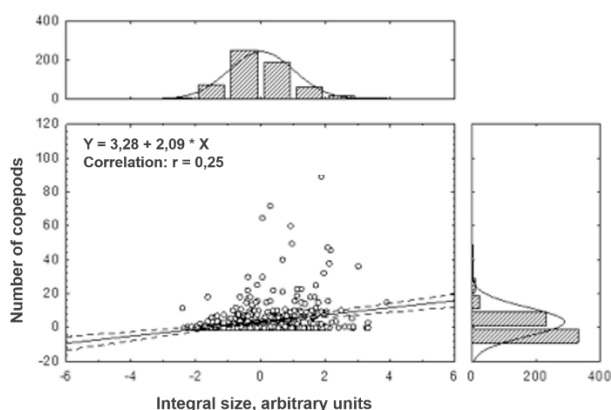


Figure 1: The connection of the integral size (calculated during the factor analysis) of starfish *A. rubens* and the number of copepods *S. gibberum*; on top – the histogram of size classes of starfishes; on the right – the histogram of distribution of starfishes by the number of copepods

Figure 1 shows a scatter diagram for the dependence of "integral size index - the number of copepods" and histograms for these characteristics. An isolated class of medium size individuals had a relatively greater number of copepods, with

the boundaries of - 0.5 - 2.2, which corresponds to bands Hr 20.9-32.50 mm., R 14.9-23.9 mm, R 52.1-80 mm. For copepods it is typical to have negative binomial distribution on the surface of starfishes.

3.2 Spatial differences in the population

Using the values of intensity, extensiveness, it is possible to select points with high indices of the population - in Polovie islands and "Babie Sea". The lowest value is typical for the area of Eremeevskie Islands and Chernaya river. Using the values of the variation index the same group of points were distinguished (Figure 2). In the area of Eremeevskie islands only one adult *S. gibberum* female was found on the surface of starfishes.

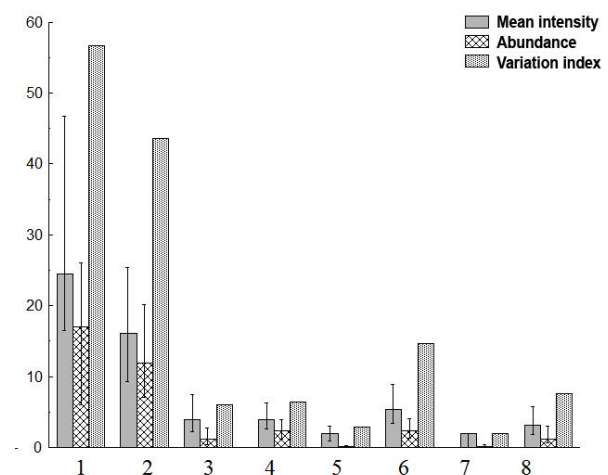


Figure 2: Spatial differences of the population of starfish *Asterias rubens* by copepods *Scottomyzon gibberum* 1 – the "Babie Sea", 2 - Polovie islands, 3 - Black gap-Ermolinskaya bay, 4 - MSU WSBS pier, 5 - in the area of Eremeevskie islands, 6 – Kislaya bay, 7 – Chernaya river, at the entrance to the bay, 8 - Krestovie islands

3.3 Distribution of copepods on the surface of the starfish *Asterias rubens*

Copepods mostly live on the lateral side of the rays and the oral side of starfishes (Figure 3). Our observations have shown that pedicellariae which are greater on the aboral side are able to capture and hold the copepods while moving planktonic stages are settling on the surface of starfishes. Depending on

the ratio of single individuals and individuals in galls the Babie sea points stand out.

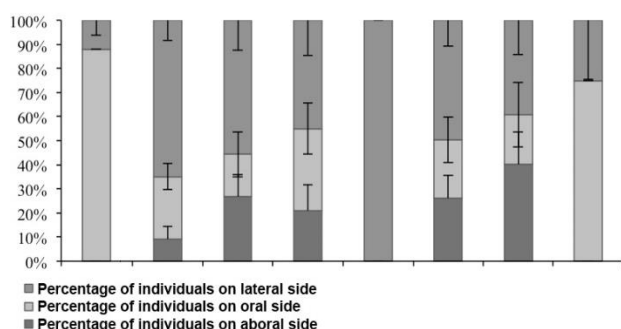


Figure 3: Percentage of individuals of *Scottomyzon gibberum* on different surfaces of the starfish *Asterias rubens* body and the number of single individuals. 1 – the "Babie Sea", 2 - Polovie islands, 3 - Black gap-Ermolinskaya bay, 4 - MSU WSBS pier, 5 - in the area of Eremeevskie islands, 6 – Kislaya bay, 7 – Chernaya river, at the entrance to the bay, 8 - Krestovie islands

Seasonal dynamics in increase or decrease of the individuals' proportion in the galls and the distribution of copepods on the body surface of starfishes were not observed for any of the stations across the studied area.

3.4 The number of cells in the starfish coelomic fluid

Both the number of cells and the proportion of micronuclei in cells are different in starfishes collected from different stations. There are two points in Polovie Islands ($77.8 \pm 15.6 \times 10^5$ cells/ml) and in the "Babie Sea" ($86 \pm 10.6 \times 10^5$ cells/ml) characterized by high values of the number of cells in the coelomic fluid. There are three points with relatively low values in the central areas (the average value of 35×10^5 cells/ml), the values do not differ. And there is a point in the estuary of Chernaya river ($54.3 \pm 15.6 \times 6.7^5$ cells/ml) with higher values in comparison with the mentioned above.

3.5 The area of the carapace copepods

There are differences in the size of the carapace for different points of the studied area (Figure 4). The largest copepods are observed in Kislaya bay area.

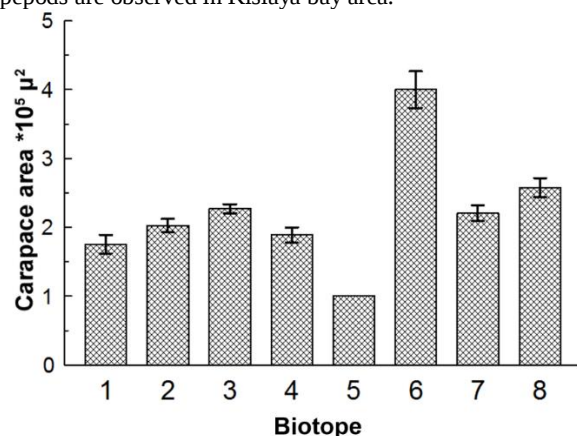


Figure 4. The area of the carapace of copepods *Scottomyzon gibberum*. 1 – the "Babie Sea", 2 - Polovie islands, 3 - Black gap-Ermolinskaya bay, 4 - MSU WSBS pier, 5 - in the area of Eremeevskie islands, 6 – Kislaya bay, 7 – Chernaya river, at the entrance to the bay, 8 - Krestovie island

4 Discussion

The study area includes several biotopes with different physical, chemical, and hydrological conditions as well as the sources and levels of anthropogenic influence. The "Babie Sea"

stands for its hydrological characteristics and processes of bioaccumulation of organic matter in sediments that reach similar levels with a major port city such as Kandalaksha (Peresyphkin, 2003). The area of Eremeevskie islands is characterized by laminaria kelp and strong currents that affect living organisms at this point. Kislaya bay is a broad sandy littoral with thickets of eelgrass. Starfishes at this point have different food ration and color. Chernaya river estuary forms a "bath" with stratified salt water, which the population of starfish living at the bottom in low salinity (21 ‰) and intense rushing river nutrients runoff. In the area of the pier of MSU Biological Station anthropogenic impact takes place due to the prolonged economic activity and intensive use of small and medium-sized boats. The salinity of the water varies within the range of 21-26 ‰. The points in the area of Lomonosov MSU biological station, Polovie islands and the "Babie sea" are located in the area with high anthropogenic load. Females of *S. gibberum* can settle on the oral, aboral and lateral sides of the starfish. When salinity is lowering the population of individuals on the aboral side increases ($t [1, 19] = 2.6, P < 0.01$). In the previous studies (Smurov, 1993) it was noted that the copepods on the aboral side are not revealed. The population depends on the age of starfishes which can be conventionally defined basing on the size. To evaluate the population of *S. gibberum* in starfish *A. rubens* the most populated "average" size class of starfishes was selected. Juveniles are almost unpopulated, big adult individuals are populated insignificantly as well. For the studied area the population is connected to the type of biotope. The highest values of the population indices are relevant for stony silty soils found in the sea near the "Babie sea" and Polovie islands. The lowest values are relevant for Krestovi and Eremeevskie islands which are characterized by thickets of laminaria and the domination of small specimens (Figure 5). The observed high values of the variation index near Polovie islands and the "Babie sea" suggest low individual resistance and poor immune response (Ieshko, 2009). In the most contaminated areas (the "Babie Sea" and Polovie islands) of the studied area the population is higher.

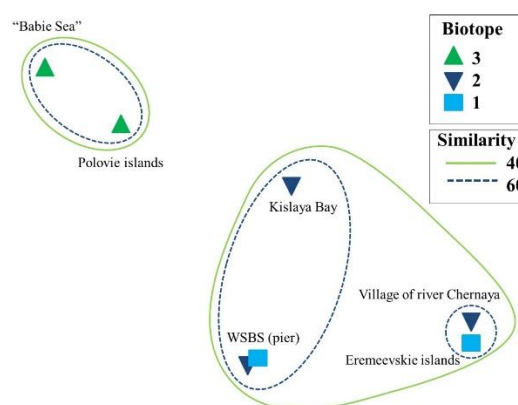


Figure 5: The spatial differences in the population of *Asterias rubens* starfish by copepods *Scottomyzon gibberum* (ordination analysis: Bray-Curtis index of similarity, cluster analysis for group average and Kruskal ordination analysis with non-metric scaling procedure (MSD Kruskal's nonmetric scaling procedure)). The types of biotopes: 1 - rocky ground with thickets of laminaria, 2 - sandy ground with eelgrass, 3 - rocky muddy ground

The conditions of biotope also affect copepods themselves. The area of the carapace is wider in the areas with low salinity ($t [1, 762] = 0.2, P < 0.01$). In the area of Kislaya bay, where there are the greatest sizes of the carapace, sandy soil is prevailing, the current speed is slow, there are Eelgrass

meadows, the starfishes differ in color (they are lighter). Sandy soil and Eelgrass meadows are characteristic also for the rest of the points where there is a relatively large area of the carapace - "black gaps", WSBS pier and Chernaya river. Starfishes differ in terms of the amount of cells in the coelomic fluid, these values are higher in the areas with a greater population of copepods ($F [2, 5] = 14.2$; $P < 0.029$) - Polovie islands and the "Babie sea". The relatively high number of cells in the coelomic fluid is also typical for the area of Chernaya river, in the population of starfishes that live in low salinity. Under experimental conditions with low salinity the toxic effect of metals is revealed through the appearance of bacterial contamination of the coelomic fluid (Poromov, Smurov, 2014).

The symbiotic association of starfishes *Asterias rubens* and copepods *Scottomyzon gibberum* Scott even in small areas have differences in the populations that may be associated with both the levels of anthropogenic load, and the features of biotopes. In this association the host and the symbiont influence each other. Starfishes affect the distribution of copepods on the surface due to the activity of pedicellariae. The increased number of copepods thus leads to the activation of immune processes with the increase in cell number in the coelomic fluid of the starfish in the most populated areas. At the same time, these processes may be pedicellariae greatly affected by both the natural features of biotopes (salinity, depth, current speed, and the type of biotope) and the anthropogenic factor.

Ethical issue

Authors are aware of, and comply with, best practice in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses and manuscript writing.

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