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RESEARCH PAPER

Comparative Characteristics of Indicesto Assess the Quality of Mussel Production by an Example of Cultivated *Mytilus galloprovincialis* (Crimea, the Black Sea)

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Abstract

Some indices (the condition index, the state index, the condition factor, the meat yield index and gonadosomatic index) were tested and compared to estimate the degree of shell filling with meat for cultivated *M. galloprovincialis* from (Crimea). The peaks of values of the the state index, the condition factor and the meat yield index had noted in September (150±8.8%, 3.6±0.2%, 35.8±2.2%, respectively). All the indices can be used as indicators of quality of mussel production. If using gonadosomatic index to defend optimal harvest time for *M. galloprovincialis* cultivated it is shown that mollusks reaches commercial quality at gonadosomatic index >8.5. The meat yield index has been proposed as easy and handy for assessing the degree of shell filling with meat. Mussels acquire optimal commercial quality if the meat yield index is more than 20%. It has been shown the total weight of ten mussels it is enough for convenient determination of the meat yield index. Harvesting time depends on environmental factors and marine farm location. It is rational to collect *M. galloprovincialis* all year round, except for December and March at the north of the Black Sea (Crimea).

Introduction

Marine bivalves have become commercially very important species because of increasing global seafood consumption in recent years (FAO Yearbook, 2017). Various physiological indices have been widely used in aquaculture of bivalve mollusks (Çelik, Karayücel, Karayücel, Eyüboğlu, & Öztürk, 2012; Vishwajeet, Lagade, Shital, Taware, & Deepak, Muley, 2015; Orban *et al.*, 2002; Zardi, McQuaid, & Nicastro, 2007; Galvao, Longo, Paulo, Torres, & Malm, 2015; Karayücel, Çelik, Karayücel, Öztürk, & Eyüboğlu, 2013; Kholodov, Pirkova, & Ladygina, 2017). They are based on the ratio of the weight and volume of the shell, wet and dried soft tissues. The indices can serve both to determine the quality of marine products and harvesting strategy and to characterize the ecological status (growth, reproduction, etc.) of cultivated mollusks (Vishwajeet *et al.*, 2015). According to various publications, both endogenous and exogenous factors -water temperature, food availability, reproductive cycles affect the physiological fitness of bivalves (Celik *et al.*, 2012; Vishwajeet *et al.*, 2015; Zardi *et al.*, 2007). Some authors have shown that the fluctuation of the indices can also be affected by pollution (Almeida, Pereira, Gomes, João Bebianno, & Cravo 2011; Rouane-Hacene *et al.*, 2015). Therefore, many authors refer these indices by “state indices” (Davenport & Chen, 1987; Celik *et al.*, 2012; Karayücel *et al.*, 2013). Most often, researchers use the meat yield index (or percentage edibility) (Orban *et al.*, 2002; Celik *et al.*, 2012; Karayücel *et al.*, 2013; Vishwajeet *et al.*, 2015). The gonadosomatic index (GSI,) can be used as an indicator of maturity of the gonads along with data of optical microscopy of gonads (Kholodov *et al.*, 2017).

Mytilus galloprovincialis Lamarck 1819 is a one of the main marine bivalve mollusk yielded commercially in the Black sea. There are two peaks of spawning of the mussel in the Black Sea: in autumn and in spring (Pirkova, Stolbova, & Ladygina, 1995). The onset of spawning and its duration are mainly determined by the environmental conditions and especially by water temperature. Winter spawning of mussels is also possible if favorable weather has established (Ivanov *et al.*, 1989). Thus, it is not feasible to exactly estimate time of *M. galloprovincialis* spawning because this process depends on the climatic conditions and feeding (Ivanov *et al.*, 1989; Pirkova, Kholodov, & Ladygina, 1999; Kholodov *et al.*, 2017). For this purpose, it is necessary to regularly monitor meat content in farmed mussels. Sometimes the abundance of fodder phytoplankton is one of the factors limiting the growth and reproduction of cultivated bivalve mollusks (Ivanov *et al.*, 1989; Ivanov *et al.*, 2007; Orban *et al.*, 2002). The food spectrum of mussels includes microalgae up to 80 microns in size (Ivanov *et al.*, 2007), the largest feed value represented by algae classes Dinopyta, Haptophyta, as well as small-cell Bacillariophyta.

To date, there is no single universal index for assessing the quality of mussel harvest, so the aim of our investigation is to test and compare indices selected from literature for *M. galloprovincialis* cultivated in the coast of the Crimea (the Black Sea) and propose the most optimal index for farmer.

Materials and Methods

Mussels of market size ≥50 mm were collected monthly at the farm located in the outer of Sevastopol Bay (44°37'13.4" N, 33°30'13.6" E) from a pilot rope collector (6 m depth) from June 2015 until August 2016. The CI and CI2 indices were calculated from November 2015. The measurement of the sea temperature (*in situ*, 6 m depth) was carried out using autonomous min probe SD204 «SAIV A/S Norway».

To study phytoplankton, water samples (V=1.5 L) were taken by reversing water bottle BM-48M from the surface and 6 m depth twice a month, condensed by a reverse filtration method through nuclear membranes (D_{pore}=1 µm). Phytoplankton samples were fixed with Lugol's iodine solution. Microalgae in the samples were counted in a live and concentrated drop (V=0.01 ml), in a camera (V=1 ml) using a light microscope «Jenaval». Identification of phytoplankton species was performed to the closest taxon possible –to genus, species or subspecies level– according to the following references: Proshkina-Lavrenko (1955), Krakhmalny (2011). The abundance and biomass of phytoplankton was calculated using the computer program "Plankton" (Lyakh & Bryantseva, 2001). To study the feeding, mussels collected in the field were immediately opened with a scalpel, the stomach was prepared and pipetted. The contents of the stomachs were fixed with Lugol's iodine solution and analyzed with a microscope.

For calculation of the indices, various parameters of the mussels were determined. The shell length was measured using a caliper with an accuracy of 0.1 mm. First, whole mussels were weighed; then they were opened, the intervalvular liquid was removed, and the soft tissue was separated from the shell. Next, the soft tissue was dried with filter paper and weighed. The dry weight of the soft tissue was determined on an analytical balance AXIS ANG200C after drying at 105°C. To calculate the internal volume of mussel shell, one valve was filled with water which was then transferred into a graduated cylinder, and the measured volume was multiplied by two. To calculate the GSI the gonads of mussels were excised with a scalpel and weighed. We took into account part of the gonad, which we were able to allocate as much as possible. The sex and gonad maturity stage were determined by visual examination of gonad smears using Jenaval (Carl Zeiss) microscope. For this, there were taken smears from different parts of the gonad using scalpel. The stage of gonad maturity and sex were determined on a 6-point scale: 1- relative resting stage, 2- beginning of gametogenesis, 3- active gametogenesis, 4- pre-spawning stage, 5- spawning, 6- post-natal period restructuring. Then, the mussels were sorted according to the stage of maturity. (Pirkova *et al.*, 1995).

The indices were calculated using the following formulas:

Condition index $CI = \left(W_{W,soft\,tissue} / V\right) \cdot 100*$ (Mladineo *et al.*, 2007)

where $W_{W,soft\,tissue}$ is wet weight of soft tissues (in g) and V is internal volume of the shell (in ml);

Condition index $CI2 = \left(W_{D,soft\,tissue} / V\right) \cdot 100*$ (Petrov, 1990; Mercado-Silva, 2005)

where $W_{D,soft\,tissue}$ is dry weight of soft tissue (in g);

State index $SI = \left(W_{W,soft\,tissue} / W_{shell}\right) \cdot 100\%$ (Galvao, Longo, Paulo, Torres , & Malm, 2015)

where $W_{shell}$is wet weight of shell (in g);

Condition factor $C_f = \left(W_{W,soft\,tissue} / L^3\right) \cdot 100*$ (Kholodov *et al*., 2017; Kopecka *et al*, 2006)

where L is mussel length (in mm);

$$\text{Meat yield} = \left(W_{W,\text{soft tissue}} / W_{\text{whole}}\right) \cdot 100\%$$ (Kholodov *et al*., 2017; Galvao *et al*., 2015; Filgueira, Comeau, Landry, Grant, Guyondet, & Mallet, 2015)

where W_{whole} is the weight of whole mussel (in g);

Gonadosomatic index $$GSI = \left(W_{\text{gonad}} / W_{\text{whole}}\right) \cdot 100\%$$ (Kholodov *et al*., 2017, Kopecka *et al*., 2006)

where W_{gonad} is wet weight of the gonad (in g).
* conditional unit

Monthly for analysis were used 50 - 52 mussels. Additionally, during the spring and autumn spawning periods, 50 mussels in each period were used to calculate the GSI. In this period, mussels were found at all stages of gonad maturity. The total 860 mussels were used in the study. All results of indexes are given as the mean of 50-52 separate analyses with ±CI (confidence interval). Analysis of variance (ANOVA) were used to determine the relationships between all measured parameters.

Results

The state index (IS), the condition factor (C_f), and the meat yield index for cultivated *M. galloprovincialis* were affected by the seasonal cycle (Figure 1). The salinity in the water area of the farm changed insignificantly (18.00±0.09 ‰ (17.70-18.04‰ – min and max). The temperature showed clear seasonal pattern and ranged from 8.9°C in February to 25.6°C in August 2015 and 26.8°C in July 2016. These were reflected gonadal development and the availability of food. During the annual cycle SI, C_f and meat yield index fluctuated between 40±3.2% (November) and 150±8.8% (September), 1.8±0.1% (December) and 3.6±0.2% (September), 18.8±1.5% (December) and 35.8±2.2% (September), respectively. The peaks of values of the indices had reached in September (Figure 1).

The indices of condition (CI and CI2) demonstrated similar trends during annual cycle. The monthly values of CI and CI2 ranged from 3.9±0.3% (March) to 6.4±0.5% (November) and from 25.4±1.5% (March) to 40.0±3.2% (November), respectively. The general trends of dynamics of the indices of mussel shell filling with meat were similar (Figure 1).

The values of all the indexes increased, when the gametes matured and decrease after spawning. It was shown the most part of the year the mussel gonads passed the third stage of maturity (from 21 to 86% of the mollusks in the sample during the year) (Figure 2). This stage is the longest. There was an increase of the gonad weight, the accumulation of nutrients, especially in females, during this period. From October to December 2015 the most of mollusks (41-52% of the total number of mussels in the sample) passed the fourth stage. During this period the weight of the gonad was maximal.

Gonadosomatic index (GSI) was investigated depending gametogenic activity. The GSI varied from 5.5 (stage of maturity 2), to 10.7 (stage 4) (Figure 3). The peak of values was observed in 4 stage (pre-spawning).

We can see GSI stayed also high (9.8) at stage 5 at the beginning of spawning. Besides value of the index was significant enough during the third stage (8.5). After the spawning, gonads became thin and translucent, so the minimal weight of soft tissue was marked at stage 2 (the onset of gametogenesis).

The total abundance and biomass of phytoplankton in the area of the farm varied from $8{\cdot}10^3$ to $23{\cdot}10^9$ cells·m^{-3} and from 30 to $7{\cdot}10^4$ mg·m^{-3} (Figure 4). The peak of abundance was noted in March and resulted from water blooms caused by small-celled diatom microalgae *Chaetocerossocialis*. The values were lowest in October ($25{\cdot}10^3$) and November 2015 ($16{\cdot}10^3$), August 2016 ($8{\cdot}10^3$ cells·m^{-3}). The highest value of biomass was watched in September due to the dominance of the large-cell diatom alga of *Proboscia alata*. The biomass was minimal in November 2015 (30 mg·m^{-3}), February (60 mg·m^{-3}) and August 2016 (30 mg·m^{-3}). Seasonal patterns of quantitative criteria of phytoplankton were significant different.

Bacillariophyta and Haptophyta were dominated in plankton in spring and autumn. Dinophyta forming the basis of the food base of mollusks (Ivanov *et al.*, 1989, 2007) were presented in the plankton constantly, but their abundance did not exceed 6% of the total phytoplankton in the area of the farm.

In the stomachs of mussels Dinophyta were the dominant group of microalgae. When small-cell Bacillariophyta and Haptophyta dominated in the plankton of farm, they are also found abundantly in the mussel's stomachs (Figure 5).

All tested indices have demonstrated similar trends, and they can be used as the indicators of the mussel production quality. The meat yield index is the easiest to assess the quality of the mussel production. The mussels reach commercial quality if the meat yield index is more than 20% (Figure 1). To optimize the farmer's work, 10 mussels are sufficient to determine the meat yield index.

Discussion

A seasonal variation in the indices of quality of the marine bivalve's production reflects the complex interactions between the available food, the environmental conditions and the bivalve's reproduction (Bressan & Marin, 1985; Vishwajeet *et al.*, 2015).

The spring spawning of *M. galloprovincialis* begins when the water warms up to the temperatures above

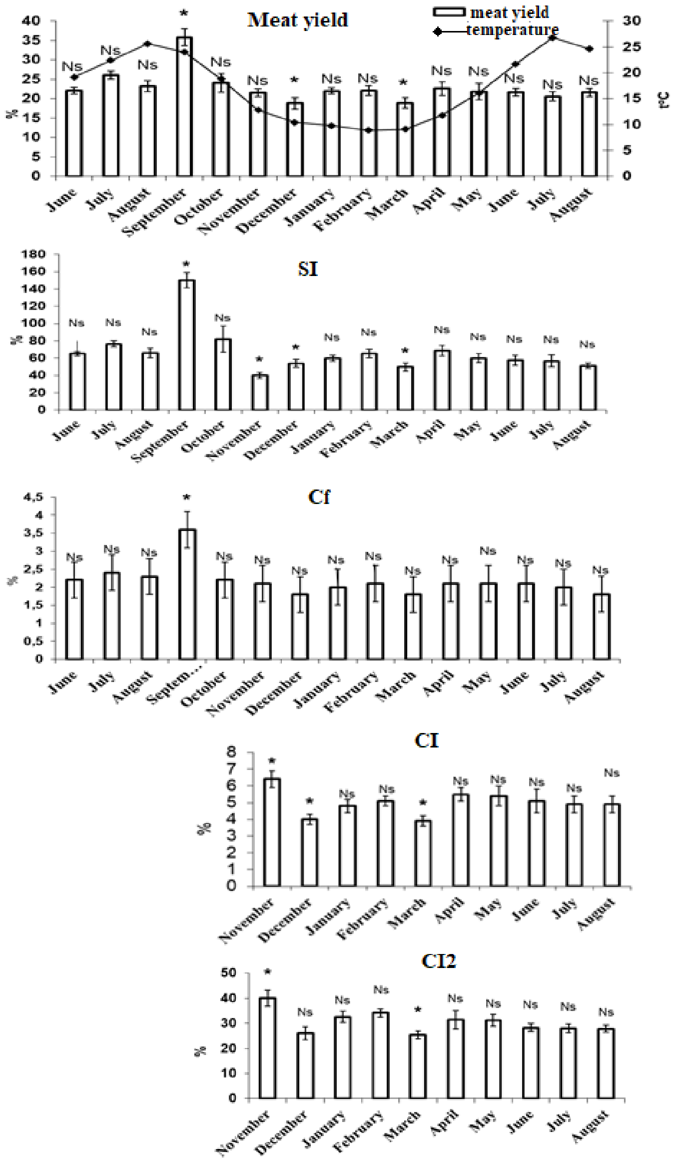

Figure 1. Seasonal dynamics of the indices of mussel shell filling with meat. Mean of n=50±CI(confidence interval). * - indicates significant difference (P<0.05), Ns - indicates insignificant difference (P>0.05).

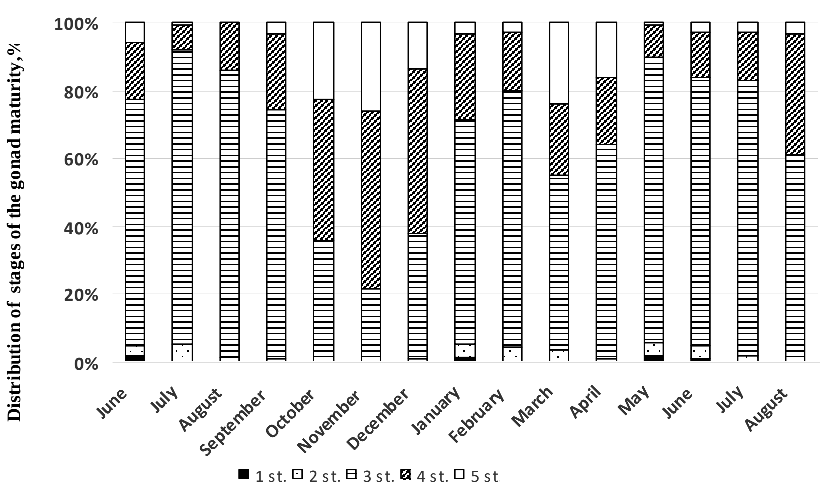

Figure 2. The seasonal dynamics of the stages of maturity of gonad mussels (2015-2016).

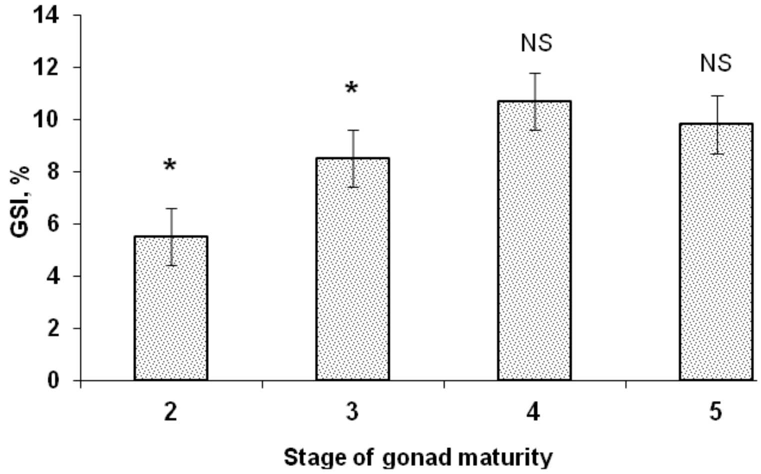

Figure 3. Change of gonadosomatic index depending on the stage of gonad maturity. Mean±CI (confidence interval). * - indicates significant difference (P<0.05), Ns - indicates insignificant difference (P>0.05).

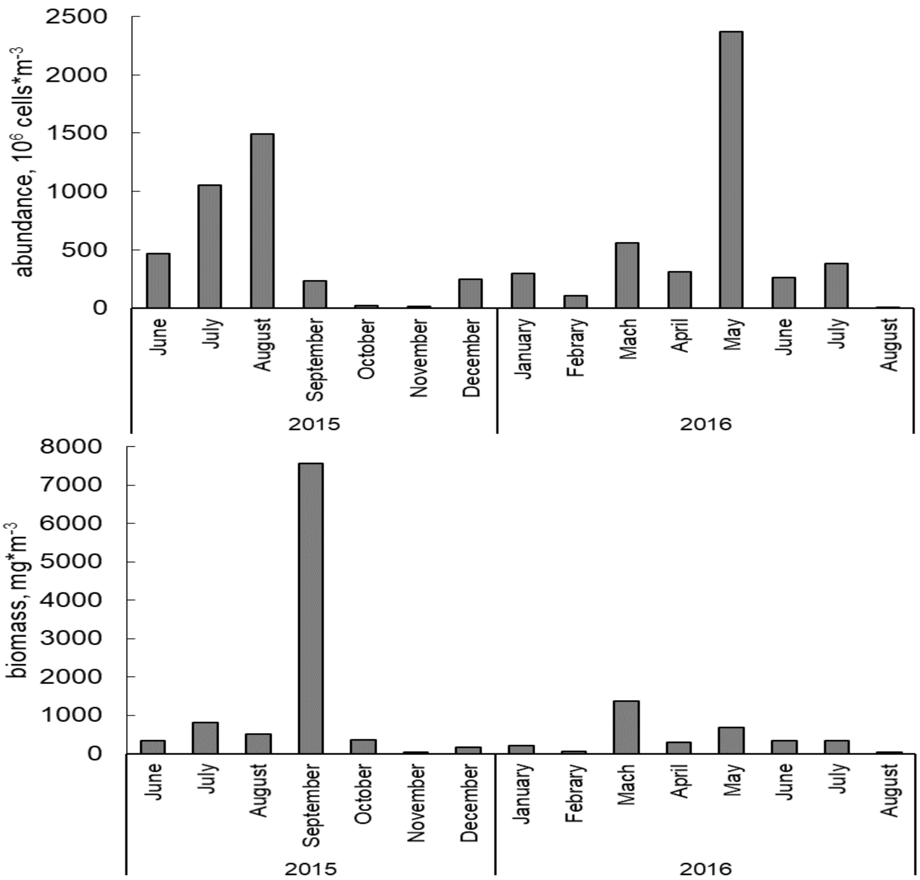

Figure 4. The abundance (A) and biomass (B) phytoplankton in the mussel farm area.

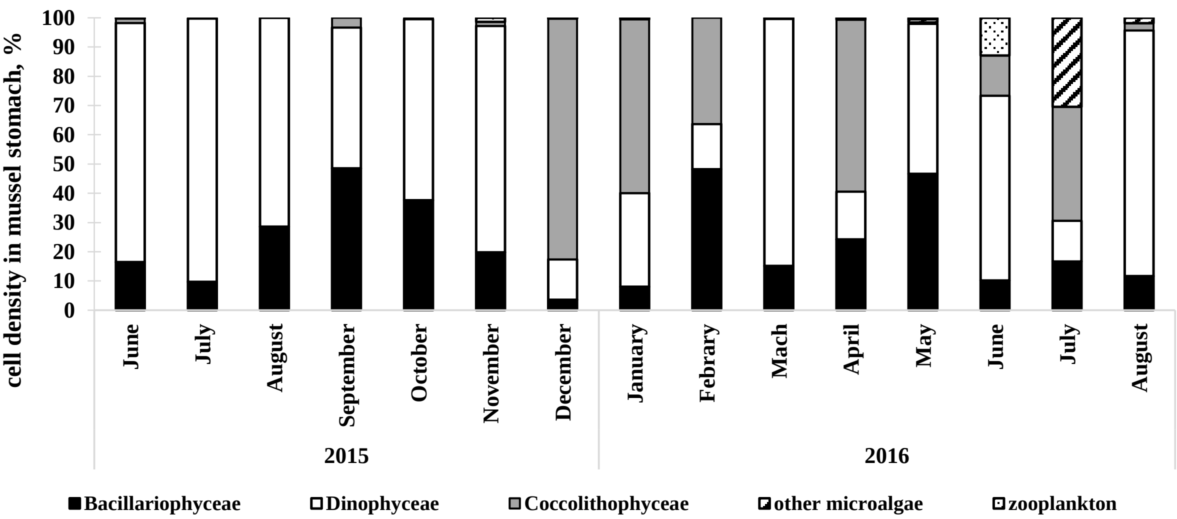

Figure 5. Numbers of organism groups in mussel stomach content.

8°C (Figure 1), and it can extend over a period of up to three months, whereas the autumn spawning begins when the temperature drops below 20°C (Figure 1), and its duration is shorter. The temperature 15-24°C is favorable for the growth of generative and somatic tissue of mussels. The growth and weight gain of mussels is reduced due to the depression of the physiological state at a water temperature above 25°C (Ivanov *et al.*, 2007; Kholodov *et al.*, 2017).

The peak values of the state index (SI), condition factor (Cf) and meat yield index in September are associated with the favorable temperature (23.4°C), gonad maturity (dominated stages – 3 and 4), and good fodder base (Figure 1, 4). In summer 2015 considerable abundance and biomass of feed phytoplankton was observed, Dinophyta algae dominated. It had a positive effect on the total weight of the soft tissue of mussels to the beginning of autumn. The water temperature in October decreased to 18.8°C, spawning commenced, and as a result, meat yield, CI, and C_f were reduced. Mollusk spawning continued in November, as confirmed by microscopic examination of gonads. In this period value of CI was the lowest. The spring spawning was registered in March and April 2016 when the water temperature was 9-12°C. From March to May, in phytoplankton of small-cell species of Haptophyta, Dinophyta and Bacillariophyta available for mussel nutrition were dominated. As the spawning period extended over more than two months, the average values of the all indices were low (Figure 1). The abundance of phytoplankton decreased in summer 2016 and reached minimum values in August (Figure 4). Because of this the filling of the mussel stomachs was low. It resulted in slight decrease of all the indices in August 2016, although mussels were on the 3rd to 4th stages of gonad maturity. The similar results were obtained in the literature. It was showed the dependence of the condition index (CI) values in *P. perna* and *Crassostrea rhizophorae* from food availability (organic seston and phytoplankton) (Narv´aez *et al.*, 2008; Rebelo, Amaral, & Pfeiffer, 2005). Good nutritional quality in farm sites has a positive influence on quality of the marine bivalve's production and gametogenesis (Çelik *et al.*, 2012). The nutritional spectrum and feed intake of mussels cultivated on the Black Sea has been investigated earlier (Ivanov *et al.*, 1989, 2007; Kholodov *et al.*, 2017). It was shown the minimum value of phytoplankton biomass for feeding of cultivated mussels should not be less than 170 mg. From our study, the biomass of microalgae in the mussel farm area was higher than 170 mg, except in November 2015, February and August 2016.

Comparing all the studied indexes, we had concluded that each of them can be used to estimate the quality of the mussel production, as they showed similar seasonal trends. Some authors suggest to use SI by shellfish farmers to assess animal health, harvest time, and yield of bivalve, since it is more easily applied (Galvao et. all, 2015). Index C_f (length/weight relationships) is often used for estimating the weight corresponding to a given length, for comparing the condition, fatness, or well-being (Vakily, 1989; Gagne *et al.*, 2008; Schmidt, O'Rourke, Hernan, & Quinn, 2011).

The GSI index is most often used to assess the reproductive characteristics of mollusks (Zardi et. al., 2007; Toro, Thompson, & Innes, 2002), and some authors suggest using it to assess the quality of cultured bivalve production (Kholodov *et al.*, 2017). We consider the using of the GSI to assess the quality of mussel production is inconvenient because it is rather difficult to separate gonads from the soft tissues and the technique requires a lot of samples to accurately calculate GSI. However, if this index is used to evaluate the time of harvesting of mussel it is necessary to take into account that *M. galloprovincialis* reach the commercial quality at GSI>8.5.

Among all the tested indices, the Meat yield index can be recommended to farmer for using. It is easily applied and the most representative to determine the harvest time of mussels. There are literature data (Kholodov *et al.*, 2017) on determination of the quality index of oysters, IQA, which is measured similarly to the meat yield index for mussels. The authors suggest that the farmer can take a total weight of 20 oysters for convenience. We have tested this method on *M. galloprovincialis*, using 10 and 20 samples of mussels. The values of the meat yield index in both cases are the same, difference is insignificant (P>0.05). Thus, for the index calculation it is sufficient to take the total weight of ten mussels.

The optimum times for harvesting of *M. galloprovincialis* depend on geographical location of farm. For mussels from North Adriatic there were suggested the end of autumn and winter (Peharda, ˇZupan, Bavˇcevi´c, Franki´c, & Klanjˇsˇcek, 2007), for mussels from south of the Black Sea there were suggested period between February and May (Celik *et al.*, 2012), the minimum and maximum meat yield were in April and July, respectively (Karayücel, Çelik, Karayücel, & Erik, 2010). From our study, we recommend harvest time for mussels from north the Black Sea (Crimea) when the meat yield index reaches more than 20%, which occurs all year round except for December and March. The maximum meat yield was recorded in September before the autumn spawning of mussels.

Conclusions

It has been shown that in the north of the Black Sea (Crimea) it is rational to collect *M. galloprovincialis* all year round, except for December and March. The maximum meat yield was recorded in September before the autumn spawning of mussels. These periods may shift depending on environmental factors and marine farm location; hence, regular monitoring of the content of meat in cultivated mussels is required. The meat yield index has been proposed as easiest for assessing the degree of shell filling with meat. Mussels have been shown to acquire optimal commercial quality if the meat yield index is more than 20%. It has been proven sufficient to use total weight of ten mussels for convenient determination of the meat yield index. To assess the quality of the mussel production, it is necessary to take into account the gonad maturity of the mussel and the composition and abundance of phytoplankton. When using the GSI to estimate the time of harvesting of *M. galloprovincialis*, it should be taken into account that mussels reach commercial quality at GSI>8.5.

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