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Conjoined Twinning Incidences in *Trachurus mediterraneus* (Steindachner, 1868) Eggs in Southern Marmara Sea

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Abstract

Conjoined twinning is a rarely existed gross malformation in natural conditions. The term refers to the two individuals united at any part of their bodies. The extraordinary incidences of conjoined twinning cases may reflect important problems on the marine environment. Therefore, the purposes of this study were to report the incidences of *Trachurus mediterraneus* conjoined twins in Bandırma Bay, Southern Marmara Sea and to discuss the possible causes of them to derive new research questions. Four polyembryonic individuals were collected from three stations of Bandırma Bay Ichthyoplankton Survey in June 2012. The anomalous individuals were observed at the stations, St-9, 10 and 13 (Critical Stations). St-9 was at about 20m depth contour between Karşıyaka town and Dalyan village in western coasts of bay. St-10 and 13 was off Yenice village at about 10 and 30m depth contours respectively in the eastern coasts of the bay. The surface temperature, salinity, dissolved oxygen and pH of critical stations were seemed to be normal. Therefore the pollution was considered as the most possible reason of these incidences.

Keywords: Bandırma Bay, ichthyoplankton, Mediterranean horse mackerel, polyembryony, Siamese twins.

Marmara Denizi'nin Güneyinde *Trachurus mediterraneus* (Steindachner, 1868) Yumurtalarına Ait Yapışık İkiz Gözlemleri

Özet

Yapışık ikizlik doğal koşullarda nadiren gözlenen ağır bir gelişim bozukluğudur ve vücutlarının herhangi bir kısmından birleşik olan iki bireyi ifade eder. Herhangi bir neden olmaksızın ağır gelişim bozukluklarıyla karşılaşılabilse de; bu tür gözlemler çoğunlukla kötüye giden çevresel koşullarla ilişkilendirilirler. Bu nedenle olağan dışı yapışık ikiz vakalarının varlığı denizel ortamı, balıkçılığı ve toplum sağlığını tehdit eden önemli problemlere işaret ediyor olabilir. Dolayısıyla, bu çalışmanın amacı Güney Marmara Denizi, Bandırma Körfezi'nde *Trachurus mediterraneus* türüne ait yapışık ikiz vakalarını takdim etmek ve yeni araştırma sorularını elde etmek amacıyla muhtemel nedenlerini tartışmaktır. Haziran 2012 tarihinde, Bandırma Körfezi İhtiyoplankton Taraması kapsamındaki istasyonlarda 16 horizontal ve vertikal Hensen Net çekimi gerçekleştirilmiş; bu istasyonlardan üçünde yapışık ikiz vakalarıyla karşılaşılmıştır. Tüm istasyonlar genelinde *Trachurus mediterraneus,* tüm yumurtaların %34.74'lük kısmını oluşturarak en baskın tür olmuştur. Anormal gelişime sahip bireyler kıyısal bölgede yer alan 9, 10 ve 13 numaralı istasyonlarda gözlenmiştir. Dokuz numaralı istasyon yaklaşık 20 m derinlikte olup; körfezin batı kıyılarında, Karşıyaka Beldesi ile Dalyan Köyü arasında yer almaktadır. On ve on üç numaralı istasyonlar ise körfezin doğu kıyılarındaki Yenice Köyü açıklarında yaklaşık 10 ve 30m derinliklerdedir. Her üç istasyonda gözlenen toplam tür sayısı 16 adettir. *Trachurus mediterraneus* yumurtalarının toplam bolluğu 9 numaralı istasyonda 166 adet (%34.80), 10 numaralı istasyonda 288 (%84.42) ve 13 numaralı istasyonda ise 173 (%35.02) adet olarak belirlenmiştir. Bu istasyonlarda yüzey suyundan yapılan ölçümlerde sıcaklık, tuzluluk, çözünmüş oksijen ve pH değerlerinde herhangi bir anormallik gözlenmemiştir. Dolayısıyla gözlenen yapışık ikiz vakalarının kirlilik nedeniyle meydana gelme olasılıklarının yüksek olabileceği düşünülmüştür.

Anahtar Kelimeler: Bandırma Körfezi, ihtiyoplankton, Sarıkuyruk istavrit, çok embryonluluk, Siyam ikizleri.

Introduction

Malformation is a deviation of any characters from normal developmental traits. "Conjoined" or "Siamese" twinning is could be included to this context as a quite rarely existed severe malformation (Owusu-Frimpong and Hargreaves, 2000). Both of the terms express two individuals connected at any part of their bodies (Arbuatti *et al*., 2011). Twining usually occurs due to the presence of two developmental centers arisen from one germ ring, which is known as polyembryony and has been classified according to conjoining body parts and developmental level (Laale, 1984). Anadidymus type twins have two head with a single common tail, whereas the katadidymus type has only one head and two tails. On the other hand, the case in which both of the individuals that are in same growth levels in appearance is named as “autosita”, whereas the term “parasita” refers to an underdeveloped individual on a better developed one (Laale, 1984).

Embryonic malformations are convenient indicators of deteriorating environment (Cameron *et al*., 1996). The presence of a stressor during development may be related to the increasing rate of embryonic malformation regardless of the malformation type (Westernhagen, 1988). Numerous studies have shown temperature and salinity out of the species specific optimal range (Westernhagen, 1988), thermal shocks (Owusu-Frimpong and Hargreaves, 2000), hypoxic conditions (Gercken *et al*., 2006), extreme pH (Arbuatti *et al*., 2011), chemical compounds such as triphenyltin (Zhang *et al*., 2008), heavy metals (Jezierska *et al*., 2009) and aromatic, chlorinated and petroleum hydrocarbons (Westernhagen, 1988) may cause polyembriyony in fish eggs.

Therefore, increasing rate of malformations such as conjoined twinning in fish eggs may be a considerable argument of unfavorable conditions (Westernhagen *et al*., 2001). Accordingly, monitoring and reporting the malformations and evaluation the possible reasons have great importance in respect of marine ecosystem, fisheries and even public health. Hence, the major objective of this study was to investigate the incidence of conjoined twinning in *Trachurus mediterraneus* (Steindachner, 1868) eggs in Southern Marmara Sea; and derive new research questions by discussing the effects of environmental conditions and pollution as possible reasons.

In similar with the cases reported here, several attempts have been made to state conjoined twinning incidences. Laale (1984) noted that first known conjoined twinning incidence in fishes was an anadidymus type elasmobranch reported by Ulisse Aldrovandi in 17th century; and also reviewed numerous early records of polyembryonic fresh water and marine teleost fishes. Most of the Siamese twins, which were observed in wild populations, were in embryonic or larval stages. Cable (1940) stated a two headed embryo of wild *Syngnathus floridae* from Western Atlantic. Westernhagen (1988) and Cameron *et al*. (1996) mentioned conjoined twin observations in *Gadus morhua*, *Platichthy flesus* and *Pleuronectes platessa* during egg surveys in North Sea, a highly polluted area. Al-Jufaily *et al*. (2005) observed anadidymus type larval Siamese twins in wild *Arius dussumieri* population from Gulf of Oman. Gercken *et al*. (2006) reported anadidymus type wild *Zoarces viviparus* larvae from Baltic Sea. Valcárcel *et al*. (2011) observed a twinning incidence in wild *Gambussia halbrooki* eggs in Iberian Peninsula. The cases seem to be relatively intense in culture conditions. There are records from Chiclids (Hulata and Rothbard, 1978; Huang *et al*., 1987), Salmonids (Bruno, 1990), Cyprinids (Jezierska *et al*., 2009), Poecilids (Arbuatti *et al*., 2011) and Syngnathids (Blanco *et al*., 2012) in aqua-culture environment.

Materials and Methods

The plankton samples were collected at 16 stations in Bandırma Bay, Southern Marmara Sea (40.3505ºN, 27.9072ºE; 40.4678ºN, 27.9072ºE; 40.3505ºN, 28.1662ºE; 40.4678ºN, 28.1662ºE) in June 2012 (Figure 1). The operations were conducted by towing a 500µm mesh size Hensen Net horizontally and vertically at each station. Water column from bottom to the surface was sampled in vertical tows. Average horizontal towing duration was 10 minutes. After each operation, the net was rinsed and all sample content was preserved by using 4% buffered formaldehyde for the purpose of carrying to the laboratory of Istanbul University, Institute of Marine Science and Management. The physicochemical parameters; temperature, salinity, dissolved oxygen and pH of the stations were measured by using a WTW 340i multi parameter measurement instrument.

The embryonic and larval stages were identified at the possible lowest taxon shortly after they were carefully sorted from zooplankton by using a stereo microscope, Leica M125. The ichthyoplankton specimens were identified following Dehknik (1973), Yüksek (1993) and Mater and Çoker (2004). The embryonic developmental stages were adapted to classification suggested by Westernhagen *et al*. (1988). The conjoined twins were preserved in 4% neutralized formaldehyde solution after the morphological characters were identified and recorded. Conjoined twins were classified according to Laale (1984).

The average and 99% confidence intervals of environmental variables were calculated in order to check anomalies in the natural conditions. Then, the spatial differences of temperature, salinity, dissolved oxygen and pH were evaluated by using one-way ANOVA (Sokal and Rohlf, 1995) in R 3.1 statistical environment (R Core Team, 2014). Egg diameters of abnormal individuals were also statistically compared with the normal ones by using t-tests (Sokal and Rohlf, 1995).

Results

Totally 6198 eggs in 25 species were sampled during the survey. Among them, *Trachurus*

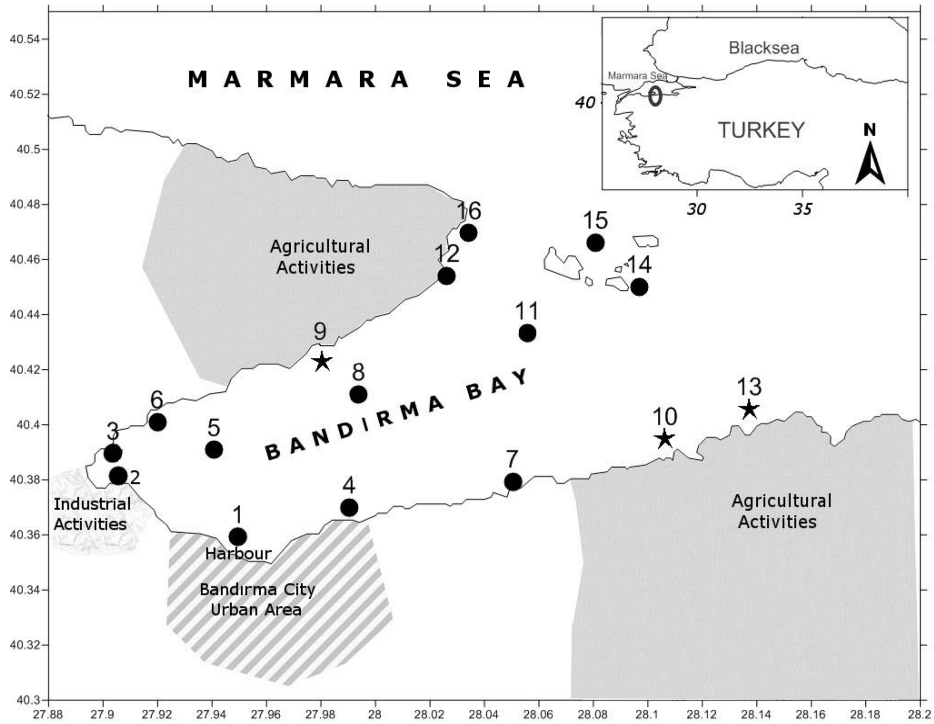

Figure 1. Geographical representation of sampling area by considering the critical (malformation observed) stations and possible pollution risks. Stars demonstrated the critical stations.

mediterraneus was the dominant species during sampling period with 2153 individuals constituting 34.74% of all eggs (Table 1). All of the individuals were at between IInd and IVth embryonic stages. The normally developed *Trachurus mediterraneus* eggs were spherical with an anterior oil droplet. The chorion was smooth and the vitellus had a heterogeneous appearance in early embryonic stages, whereas it was homogenous in late stages. The egg diameter was between 0.71 and 0.86mm (mean 0.78±0.01mm) and oil droplet was between 0.17 and 0.21mm (mean 0.19±0.01mm) in normal individuals. Double rows of punctate melanophores which extend through posterior were visible after the tail was separated from yolk (Figure 2a).

We observed four polyembryonic *Trachurus mediterraneus* eggs in the horizontal tows of Stations (St) 9, 10 and vertical tow of Station 13. Conjoined Twins 1 and 2 (CT-1 and CT-2) were collected at St-9; Conjoined Twins 3 (CT-3) was collected at St-10 and Conjoined Twins 4 (CT-4) was collected at St-13. The pooled total species number in egg stage was 16 at the critical (malformation observed) stations.

The sample CT-1 was a katadidymus-parasita type conjoined twin. The head development was normal and embryo was up to 180º around yolk (II. embryonic developmental stage). Egg diameter (0.67mm) was highly significantly smaller than those of the normal ones ($P<0.001$), whereas oil droplet diameter (0.18mm) was not different ($P>0.05$). There was not pigmentation. The embryonic development seemed to be normal until 8th somite where second, underdeveloped (parasitic) tail appeared. The parasitic tail had 9 somites without a vertebral tube. It had disorders also at the tip. The main tail seemed normally developed. It had about 15 somites and an external morphologically normal vertebral tube. The angle between two tails was about 100º (Figure 2b). CT-2 was also a katadidymus-parasita type twin. However, there were not any signs of head development such as eye cups or otic capsules; the anterior part of embryo was considered as head region because there were no somites in this part. The embryo was at the stage II. Egg diameter (0.75mm) was significantly smaller than normal ones ($P<0.001$), whereas oil droplet diameter (0.18mm) showed no significant differences with the normally developed embryo ($P>0.05$). There was not pigmentation. The parasitic tail appeared at the 10th somite. It had 8 somites without vertebral tube. The vertebral tube was in the other tail which had 10 somites and a relatively normal appearance. Developed tail was in parallel with the parasitic one (Figure 2c). CT-3 was also a katadidymus but autosita type twin. The head developmental defect was similar with that of the CT-2. It was at stage II in general appearance. Egg and oil droplet diameters (0.18mm) were not significantly different than those of normal ones ($P>0.05$). The tails were separated at the 8th somite and both of the tails had three somites (Figure 2d). Similarly with the other cases, the CT-4 was a stage II, katadidymus-parasita type conjoined twins. Egg (0.18mm) and oil droplet (0.17mm) diameters were significantly smaller than those of normal ones ($P<0.01$). Head development

Table 1. Metadata,ecological parameters and environmental conditions of survey water of survey field and critical stations.

Parameter	Overall[a]	St-9	St-10	St-13
Coordinates	-	27.97982ºE 40.42578ºN	28.10628ºE 40.39484ºN	28.13723ºE 40.40560ºN
Depth (m)	-	20	10	30
Tow	-	Horizontal	Horizontal	Vertical
N. of Spec.[b]	25	13	10	8
Abundance[c] (ind)	2153	166	288	173
Dominance[d] (%)	34.74	34.80	84.42	35.02
Temperature (ºC)	27.29±0.52	27.50	27.70	27.4
Salinity (‰)	23.92±0.21	23.90	23.90	23.9
pH	8.27±0.04	8.23	8.29	8.25
Oxygen(mg/l)	6.24±0.45	5.25*	5.75*	5.92

[a]Overall column shows total values for ecological parameters and average values for environmental parameters.[b]total number of species in egg stage. [c]number of *Trachurus mediterraneus* eggs; [d]propotional abundance of *Trachurus mediterraneus* eggs. ±valuesare 99% confidenceintervals of overallaverage of environmentalvariables. * indicates significant difference from the overall mean at 99% confidence level.

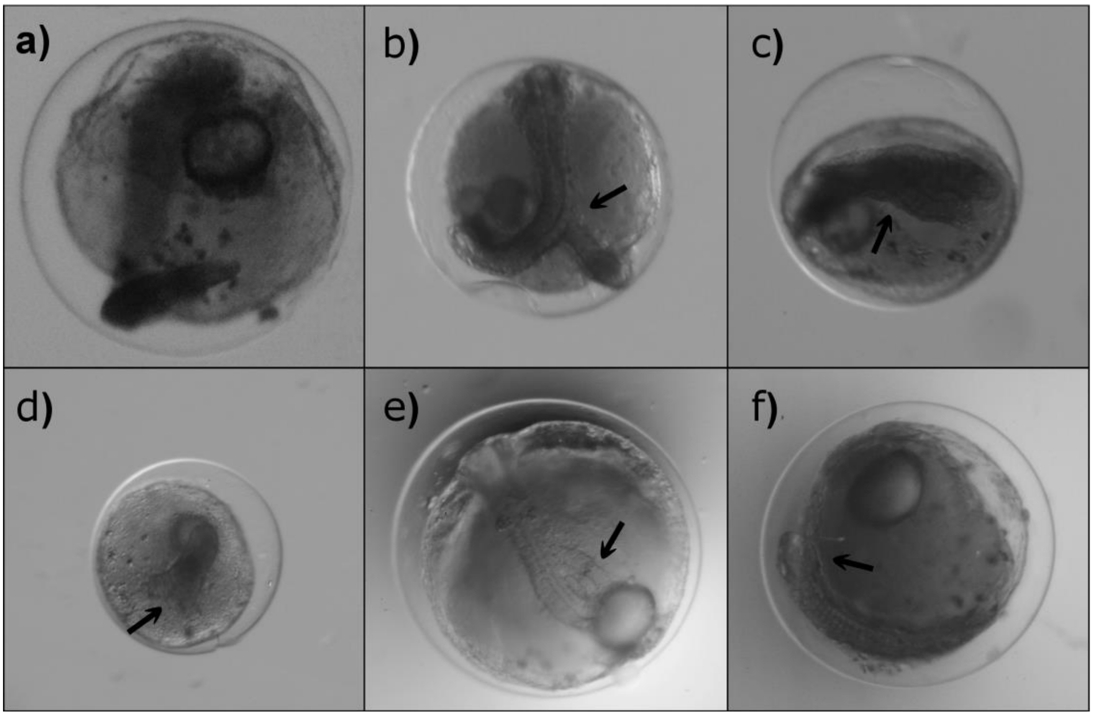

Figure 2. Comparison of normally developed *Trachurus mediterraneus* eggs (a) with abnormal incidences (b-f).

was also normal. The parasitic tail appeared at the 11th somite and had 3 somites. The main tail had 6 somites. Vertebral tube appeared in both of the tails. There was no pigmentation (Figure 2e). In addition to conjoined twinning incidences, an acephalic individual was observed at the vertical tow of St-10 (Figure 2f). The sample had 20 somites and a normal pigmentation development. The absence of eye cups in the anterior part of body suggested no head development. The egg (0.75mm) and oil droplet (0.16mm) were significantly smaller than those of the normally developed conspecifics (P<0.01).

Table 1 compares overall average with the sea surface physicochemical conditions of critical stations. The spatial changes of environmental conditions were not significant (ANOVA; P>0.05). There were not significantly anomalous physicochemical conditions at the stations where the conjoined twins were obtained except for oxygen. St-9 and St-10 had slightly but significantly lower surface oxygen values than overall mean (P<0.01).

Discussion

Malformations such as conjoined twinning are important indicators of quality and developmental capacity of fertilized fish eggs (Kjorsvik *et al*., 1990). Substantially, most of the malformed individuals cannot survive until hatching (Westernhagen *et al.*, 1988). This fact is more dramatic in severely malformed specimens such as conjoined twins under the natural conditions (Bruno, 1990). In this sense, enhanced mortality in early stages of fishes is able to affect the year class strength of fish populations significantly (Gallego *et al.*, 2012).

However, the usage of size as a quality indicator is questionable (Brooks *et al*., 1997); there are clear evidences showing that malformed individuals significantly smaller than normal ones (Blanco *et al*., 2012; Llanos-Rivera *et al*., 2013). These results are consistent with our observations and may indicate low egg quality.

There are reports demonstrate that temperature, salinity, pH and oxygen may cause developmental anomalies (Westernhagen, 1988; Gercken *et al*., 2006; Arbuatti *et al*., 2011). However, we did not observe significantly different or anomalous conditions of environmental variables at critical stations (St-9, 10 and 13). On the other hand, the oxygen values were significantly lower than overall mean at St-9 and 10; however, they are higher than 2mg/l which is critical biological limit for normal embryonic development of fishes given by Westernhagen *et al*. (1988), Westernhagen (1988) and the references therein. It is considered that, mentioned arguments decreased the possibility of the oxygen was a main stressor on the anomalous occurrences.

Clear evidences show the increment of egg malformations in polluted waters in North Sea (Cameron and Westernhagen, 1997), Baltic Sea (Westernhagen *et al*., 1988), Australian Coastal Waters (Klumpp and Westernhagen, 1995), Chilean Waters (Llanos-Rivera *et al*., 2013) and Western Coasts of Atlantic (Longwell *et al*., 1992). Heavy metals and especially Cadmium (Cd) are among the important sources of developmental defects in fish embryos (Westernhagen, 1988). Mulayim *et al*. (2012) noted that anthropogenic originated Cd, Pb and Hg values in surface sediments of Bandırma Bay is higher than the average and particularly the area is defined as considerable contaminated by Cadmium. Sur *et al*. (2010) noted that Hg is also an important contaminant for the area having a contamination factor of about 13 and the area can be considered as highly contaminated by heavy metals with a contamination degree of 19. Bandırma Bay is also found to be intermediately polluted by the total petroleum hydrocarbons in surface sediments (Sur *et al*., 2010). Therefore pollution might be hypothesized an important source of environmental stress on the occurrence of the anomalous incidences also in the studying area.

Marine traffic, urban wastes, industrial facilities and agricultural activities are among the possible pollution resources in Bandırma Bay. Mulayim *et al*. (2012) attributed the heavy metal pollution to the industrial and urban wastewaters. On the other hand, the critical stations in our study were around agricultural areas and far from the industrial and urban waste water sources (Figure 1). Even though this argument suggested that the agricultural pollution was the most probable reason of the observed developmental abnormalities, the dynamic properties of marine environment such as passive drifting should not be overlooked (Peck *et al*., 2012). To clarify these gaps, further studies are needed.

The total species number in embryonic stages was 16 at the stations where conjoined twins were observed; however, the incidences only existed in *Trachurus mediterraneus*. Although it is not possible to derive actual reason of this in the current study, we may argue on some possible occasions. *Trachurus mediterraneus* was the dominant species of sampling period (34.74%). Therefore, the incidences might be attributed to the relative abundance of this species. In this instance, when the sampling intensity increases, malformations are expected to appear also in other species. Besides, according to Dionísio *et al*. (2012), the sensitivity of fishes to pollutants or to the other stressors seems to be species specific. Bobe and Labbe (2010) explain this interspecific variation with different resistance levels of genetic materials of fishes. Another explanation may be the species specific vertical distribution of fish eggs (Bunn *et al*., 2000). The parental condition and genetic heritage could be considered as another statement for the developmental anomalies (Brooks *et al*., 1997) in only one species. Furthermore, the effect or effects causing malformations may arise from parents by the way of the vitellus, and/or from the habitat to which released eggs contacted directly (Bunn *et al*., 2000). Thus, malformations may reflect the interactions of the conditions of both stages.

In conclusion, embryonic malformations may reflect the presence of a stressor or antagonistic and synergistic effects of stressors without indicating the type of them (Westernhagen, 1988). On the other hand, the fact should be insistently stated that a low amount of malformations in fish eggs may appear completely coincidently (Westernhagen and Dethlefsen, 1997). But, the evaluation of malformations by considering all the possible causes may be a reasonable approach. Bandırma Bay is an important fishery and recreational area in Marmara Sea. The polyembryony cases may indicate a serious pollution problem that may thread the ecosystem. The available data may show the problem without details and highlight some research priorities that may clarify developmental abnormalities.

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