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Original Research Articles

Observation on embryonic development of *Gymnodiptychus dybowskii* (Kessler, 1874) in Manas River, Xinjiang, China

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To protect the germplasm resources of *Gymnodiptychus dybowskii* (Kessler, 1874), a species endemic to Xinjiang, the key stages of embryonic development of *G. dybowskii* were studied. From May to July 2021, the artificial breeding of *G. dybowskii* was carried out at the fish breeding station of the Kenswart Water Conservancy Project. The embryos and early larvae were obtained using temperature promotion, water stimulation, hormone induction, and dry insemination techniques. their development process was observed, and morphological characteristics of each developmental stage were recorded. The results showed that the mature oocyte was round, heavy, and light yellow, with an egg diameter of (2.32 ± 0.21) mm. The fertilized egg was slightly viscous, and the viscosity disappeared after water absorption. Under the temperature of 15.6-17.7 ℃ (16.23 ± 0.52) ℃, the embryonic development of *G. dybowskii* lasted for 142 h and 33 min and went through 7 stages of fertilization, cleavage, blastula, gastrula, neurula embryo, organ formation, and membrane hatching, with a total of 26 periods. The membrane broke after 2376.724 ℃·h. The embryonic development of *G. dybowskii* follows the rule of freshwater bony fish, but it differs from other *Schizothorax* species in the muscular effect period and membrane emergence mode. In addition, differences in hatching water temperature among different geographic populations lead to differences in embryo development duration and accumulated temperature, which further proves that *G. dybowskii* is highly adaptive to the ecological environment of its habitat water, and these characteristics together constitute its survival and reproduction strategy in the natural environment. In this study, through the artificial breeding experiment of *G. dybowskii*, the characteristics of its embryonic development were explored, which laid the foundation for the cultivation of unique fish seedlings, enriched the early life history data of *G. dybowskii*, and further provided basic data for its resource protection.

INTRODUCTION

Gymnodiptychus dybowskii (Kessler, 1874), belonging to Cypriniformes, Cyprinidae, Schizothoracids, *Gymnodiptychus* Herzenstein,[1] is a typical rare and unique cold-water fish in China, widely distributed in the Ili River in Xinjiang, and in the north slope of Tianshan Mountains.[2,3] In 2004, 2019, and 2022, it was listed as the Xinjiang Uygur Autonomous Region's Level I key protection aquatic wildlife.[4]

In recent years, studies on *G. dybowskii* have focused on genetic diversity,[5,6] individual biology, [7-9] population distribution[10,11] and phylogeny.[12,13] Related scholars[14-16] studied the embryonic development of *G. dybowskii* in the Kaidu River and Ili River of Xinjiang. Due to the aggravation of overfishing, water conservancy construction, and other factors, the population resources of G. dybowskii and other Indigenous fish are increasingly depleted, and the population is extremely scarce.[17] Since *G. dybowskii* is distributed in different basins, there may be great heterogeneity and obvious evolutionary characteristics. Therefore, it is urgent to carry out relevant studies on germplasm conservation, proliferation, and breeding of *G. dybowskii*, especially on artificial breeding of different geographical populations, to provide technical support for the large-scale production of fish larvae. It is of great significance for the conservation and cultivation of this rare fish. By observing and studying the embryonic development of *G. dybowskii* in Manas River and comparing it with other fishes of Schizothoracinae, this study aims to find out the typical characteristics of its embryonic development at various stages, strengthen

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Table 1. Parental information of *Gymnodiptychus dybowskii*

Sex	Sample number	Body length/mm	Body weight/g
Female	6	253.12 ± 14.87	210.00 ± 14.14
Male	4	253.12 ± 14.87	210.00 ± 14.14

the conservation strategies and breeding and release practices of this unique indigenous species in the Manas River, and ensure the sustainable utilization of resources of this rare species and the maintenance of ecological balance.

MATERIALS AND METHODS

SAMPLE COLLECTION

From May to July 2021, the parents of *G. dybowskii* with strong physique and no body surface injuries will be selected for artificial breeding at the fish breeding station of Kenswart Water Conservancy Project in Manas River, Xinjiang. According to Wang[18] and Wu et al.,[3] male secondary sex characteristics were obvious, the head and male fin "beads" were obvious, and the reproductive pores were slightly protruding. The abdomen of the female fish is enlarged, soft, and elastic, and the reproductive pores are red and prominent. The parental information of *G. dybowskii* is shown in **Table 1**.

ARTIFICIAL INSEMINATION AND INCUBATION

Dry insemination was carried out with a male-to-female ratio of 1:2 ~3 when the parents of G. dybowskii were mature. After complete insemination, the fertilized eggs were rinsed with water for 10 ~ 20 min until the egg membrane absorbed water and expanded, and hatching began. The water temperature was kept above 8.00 mg/L, and it was 15.6 ~ 17.7 ℃ (16.23 ± 0.52) ℃.

The incubation facility is a self-made 0.40 m × 0.30 m × 0.10 m incubator frame, sewn with 40-mesh screen silk and placed in a 4.00 m × 0.80 m × 0.50 m flume. The fertilized eggs are laid on the screen in the hatching box with a density of 2 ~ 4 grains /cm2, and they are stirred gently once a day for 5 ~ 6 h to maintain sufficient dissolved oxygen and prevent accumulation and death. Dead eggs are picked and removed twice in the morning and evening to prevent mildew.

ARTIFICIAL INSEMINATION AND INCUBATION

During embryonic development, 10-15 fertilized eggs were randomly selected with a straw every 1 h and placed in a petri dish with water for incubation. The eggs were observed under an anatomical microscope (SMZ-140 N2GG, MOTIC, Xiamen) and a camera (PowerShot A3000 IS, Canon, Xiamen). The sequence of embryo development and morphological changes at each stage were recorded. It was observed every 20-30 min before the blastocyst stage and every 1 h during the organ differentiation stage, and more than 50% of the observed individuals showed new features as the criteria for dividing developmental periods.[19,20]

DATA ANALYSIS

Photoshop CS6 for image processing. Estimation of effective accumulated temperature at each stage of embryonic development[21]:

$$K = NT$$

Where K is the accumulated temperature (℃·h), N is the time required for development to a certain stage (h), and T is the average water temperature (℃) at the development stage. SPSS 18.0 and ORIGIN 9.0 were used and expressed as Mean and standard deviation (Mean ± S.D.).

RESULTS

The embryonic development process of *G. dybowskii* was divided into 7 stages, namely fertilization, cleavage, blastula, gastrula, neurula embryo, organ formation, and membrane hatching, with a total of 26 periods (**Table 2**).

MORPHOLOGICAL CHARACTERISTICS OF MATURE EGGS

The eggs of *G. dybowskii* were heavy, large, round, yellowish, with an egg diameter of (2.32 ± 0.21) mm (**Figure 1-1**). They are slightly viscous and swell when exposed to water. After fertilization, the viscosity of the egg was enhanced, and the viscosity was gradually weakened and the eggs dispersed. At about 0.71 h after fertilization, the height of the polar eminence of the blastoderm animal was about 1/4 of the yolk (**Figure 1-2**).

CLEAVAGE STAGE

At about 1.14 h after fertilization, a division groove appeared in the raised blastoderm, and two similar cells divided, that is, the 2-cell period (**Figure 1-3**). Embryo development is accelerated, and another division groove perpendicular to the first division groove appears, dividing cells into balls, that is, the 4-cell period (**Figure 1-4**). Then, the two divisions divide in parallel once, forming 2 columns of 4 in each column, an 8-cell period (**Figure 1-5**). Continue to divide, the cell size is uneven and irregular arrangement, that is, 16-cell period (Figure 1-6). At the 32-cell period, the individual differences of the mitotic bulb became larger (**Figure 1-7**). During the 64-cell period, the 6th division, the division time was different, and the spatial distribution and shape characteristics of blastomes were different (Figure 1-8). The division continues, with increased mitotic balls and elevated accumulation above the yolk, resulting in a Multi-cell period (**Figure 1-9**).

BLASTULA STAGE

About 10-15 h after fertilization, the cells continue to divide, the number increases, and the blastoderm eminence is obvious, which is the early blastula period (**Figure 1-10**).

Table 2. Embryonic development of *Gymnodiptychus dybowskii*

Developmental stage	Brief characteristics	Time after fertilization / h	Time duration / h	Figure ordinal
Fertilized egg	Pale yellow, round, transparent, sedate eggs	0	0	Figure 1-1
Blastoderm formation period	Animal extremes form a raised blastodisk	0.71	0.71	Figure 1-2
2-cell period	There is a longitudinal fissure at the top of the blastocyst, and 2 cells are separated	1.14	0.43	Figure 1-3
4-cell period	Four cells of similar size	3.01	1.87	Figure 1-4
8-cell period	Eight cells of similar size	3.6	0.59	Figure 1-5
16-cell period	16 similar-sized, shrunken cells	5.06	1.46	Figure 1-6
32-cell period	The cells were divided into 32 irregular cells	5.83	0.77	Figure 1-7
64-cell period	The cells were divided into 64 irregular cells	8.33	2.5	Figure 1-8
Multi-cell period	The cells continue to divide and the clumps swell	10.12	1.79	Figure 1-9
Early blastula period	Blastocyst protrusion concentrated into a "high hat" shape, the number of cells increased sharply, the boundary is not obvious	13.64	3.52	Figure 1-10
Mid-blastula period	Protruding is obvious, the size of both sides is basically equal	18.45	4.81	Figure 1-11
Late-blastula period	The blastocyst spreads along the surface of the egg toward the plant pole	19.28	0.83	Figure 1-12
Early gastrula period	1/3 of the hypoderm is enclosed, and a ring of the embryo appears, enclosing part of the yolk	27.25	7.97	Figure 1-13
Mid-gastrula period	The hypoderm encloses 1/2 and the blastocyst appears	32.18	4.93	Figure 1-14
Late-gastrula period	The germ layer encloses most of the yolk and the embryo shield elongates	42.85	10.67	Figure 1-15
Neurula embryo period	The embryo body clings to the yolk sac and the front end expands to form a brain bubble	47.53	4.68	Figure 1-16
Blastopore closing perio	The hypoderm is enclosed and the blastocyst is closed	50.13	2.6	Figure 1-17
Metameres appearance	Multiple pairs of distinct sarcomere	56.52	6.39	Figure 1-18
Eye sac formation	Both sides of the head oval depression, clear eye sac	68.20	11.68	Figure 1-19
Caudal bud appearance	The posterior end of the embryo body elongates and the yolk sac shrinks to form the tail bud	76.67	8.47	Figure 1-20
Muscular effect	The tail bud of the embryo body becomes longer, and the back and tail begin to transmit muscle movements irregularly	81.17	4.5	Figure 1-21
Lens appearance	Visible to the eyes, the embryonic body twisted frequently, and the movement mode was whole body movement	82.53	1.36	Figure 1-22
Otoliths formation	There are two granulosa in the two lateral auricular sacs of embryo body	83.70	1.17	Figure 1-23
Heart beating	The heart beats from weak to strong, and the tail stalk becomes longer and can reach the head	88.73	5.03	Figure 1-24
Hatching	The embryo body comes out of the membrane and moves violently	106.70	17.97	Figure 1-25
Newly hatched larvae	The whole body of yolk sac larva is transparent	142.55	35.85	Figure 1-26

The height of the blastocyst decreases, which is the mid-blastula period (Figure 1-11). As the blastocyst expands to

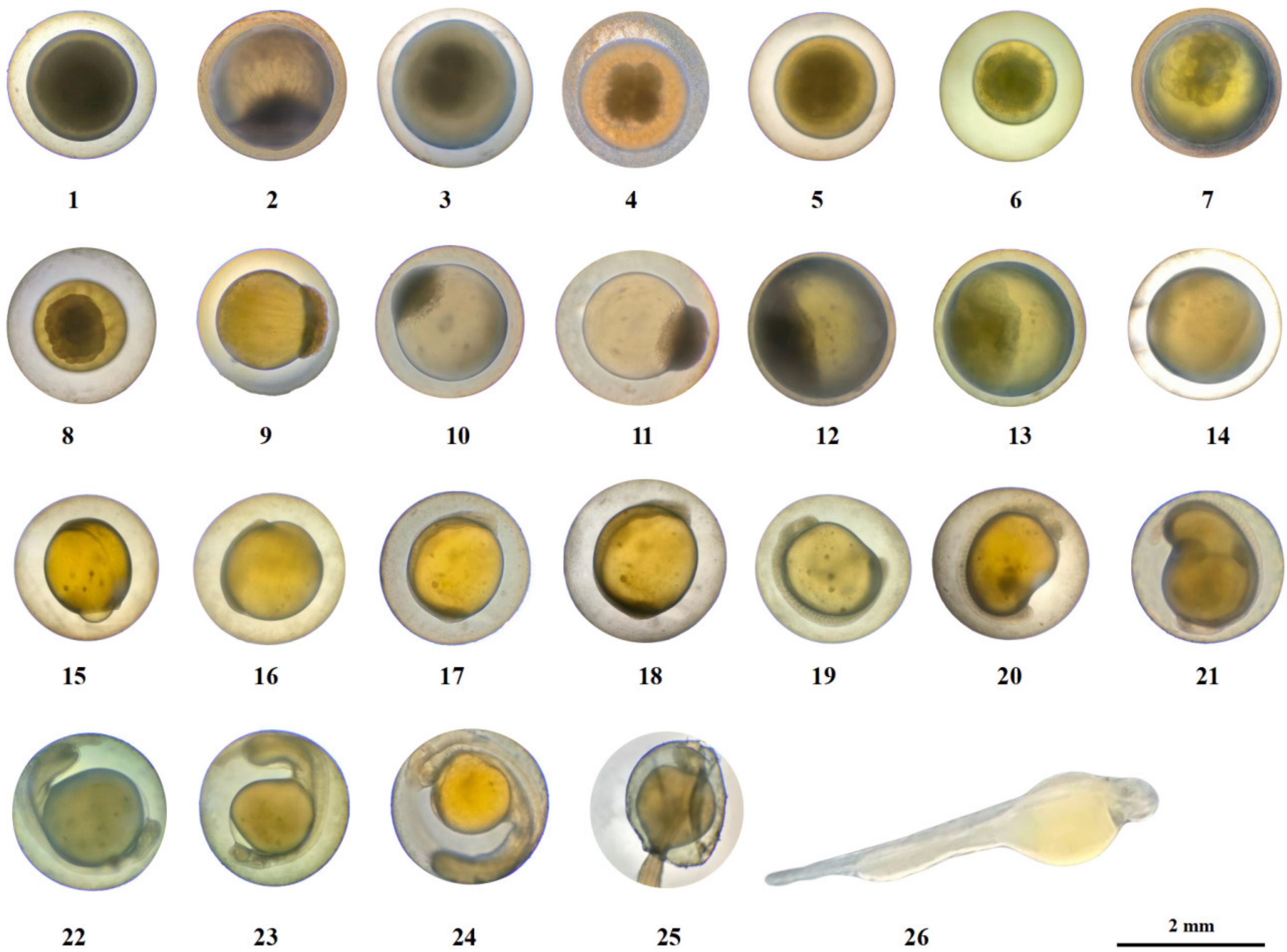

Figure 1. Embryonic development of *Gymnodiptychus dybowskii*

1. Fertilized egg; 2. Blastodisc formation period; 3. 2-cell period; 4. 4-cell period; 5. 8-cell period; 6. 16-cell period; 7. 32-cell period; 8. 64-cell period; 9. Multi-cell period; 10. Early blastula period; 11. Mid-blastula period; 12. Late-blastula period; 13. Early gastrula period; 14. Mid-gastrula period; 15. Late-gastrula period; 16. Neurula embryo period; 17. Blastopore closing period; 18. Metameres appearance; 19. Eye sac formation; 20. Caudal bud appearance; 21. Muscular effect; 22. Lens appearance; 23. Otoliths formation; 24. Heart beating; 25. Hatching; 26. Newly hatched larvae

the edge, the blastoderm shrinks and appears as a "cap" over the yolk sac, i.e., the late-blastula period (**Figure 1-12**).

GASTRULA STAGE

About 20 to 30 h after fertilization, the blastocyst cells are wrapped to the bottom of the plant to 1/3 of the yolk height, and the cell edge is curled into a blastocyst ring, which is the early gastrula period (**Figure 1-13**). Cells on one side of the ring rise to form a blastoshield, the mid-gastrula period (**Figure 1-14**). The front end of the blastoderm expands and further extends to form a larger yolk plug, which is the late-gastrula period (**Figure 1-15**).

NEURULA EMBRYO STAGE

About 35-48 h after fertilization, the subderm is covered with yolk 5/6, the plant has very little yolk, the "horseshoe" brain vesicle is formed, and the whole embryo body lies on the yolk sac, which is the neurula embryo period (**Figure 1-16**). Egg yolk is enclosed under the germ layer, and the blastopore is closed, which is the blastopore closing period (**Figure 1-17**).

ORGAN FORMATION STAGE

About 56.52 h after fertilization, 2 ~ 4 pairs of body segments were observed in the middle of the embryo body, which gradually increased, that is, the metameres appearance period (**Figure 1-18**). At 68.20 h, the length of the embryo body was extended with the increase of myotome, and the outline of the eye sac was formed, that is, the eye sac formation period (**Figure 1-19**). 76.67 h, the yolk sphere was depressed, the tail separated from the yolk sphere, and entered the caudal bud appearance period (**Figure 1-20**). At 81.17 h, irregular contractions of the back myotome and tail swaying occurred at a frequency of about 7-9 times /min and then gradually accelerated, that is, the muscular effect period (**Figure 1-21**). At 82.53 h, the eyes were visible, the embryonic body twisted frequently, and the movement mode was whole body movement, which was transferred from the head to the tail, that is, the lens appearance period (**Figure 1-22**). At 83.70 h, a pair of "stone" -like particles appeared in the ear sac of the embryonic body, which was the otoliths formation period (**Figure 1-23**). At 88.73 h, there were ventricular vacuoles at the head of the embryo body and yolk sac, with heart beating and transparent blood, that is, the heart beating period (**Figure 1-24**).

MEMBRANE HATCHING STAGE

About 106.70 h after fertilization, the larval fish exhaled the membrane and entered the hatching period (**Figure 1-25**). Before the emergence of the membrane, the embryonic body rolls or rotates faster and impinges on the egg membrane. The egg membrane becomes soft and thin, breaks near the tail, free into the water and swings, then twists violently, the break becomes larger, and the embryonic body completely emerges from the membrane (**Figure 1-26**). The newly emerging membranous larva is light yellow, with distinct body segments and no body pigment formation. The yolk sac is spherical at the front and club-like at the back, close to the abdomen of the larvae. The hemoglobin on yolk was visible to the naked eye, but the pigment of eyeball, body pigment, pectoral fin and gill were not. Larvae lie on the bottom side for most of the time, and move up or up intermittently.

According to morphological characteristics, the embryonic development process of *G. dybowskii* can be divided into 7 stages: fertilization, cleavage, blastula, gastrula, neurula embryo, organ formation and membrane hatching, with a total of 26 periods. When the water temperature was 15.6 ~ 17.7℃, the period from fertilized egg to hatching lasted 142.55 h, and the accumulated temperature was 2376.72 ℃·h. The cleavage period lasted 9.41 h, and the accumulated temperature was 150.92 ℃·h. The blastula period lasted 9.16 h, and the accumulated temperature was 143.90 ℃·h. The gastrula period lasted 23.57 hours and the accumulated temperature was 375.74 ℃·h. The duration of neurula embryo period was 7.28 h, and the accumulated temperature was 116.73 ℃·h. The duration of organ differentiation was 38.6 h, and the accumulated temperature was 636.02 ℃·h.

DISCUSSION

SIMILARITIES AND DIFFERENCES IN EGG CELL COLOR WITH OTHER SPECIES

Schizothoracinae generally inhabit plateau waters, and are commonly found in the slow flow of wide valley channels or the rapids of canyon channels,[22] and have strong adaptability to extreme plateau environments in terms of morphology and ecology. In this study, the eggs of *G. dybowskii* were observed to be yellowish in color, similar to those of *S. pseudaksaiensis*,[23] *S. wangchiachii*,[24] *S. prenanti*[15] and *S. davidi*[25]; Like *Schizothorax* species, *Triplophysa* is a genus of Cypriniformes that live at high altitudes. The egg color of *T. yarkandensis*,[26] *T. angeli*[27] and *T. leekeri*[28] were light yellow. This common trait may be the result of evolutionary adaptations to clear water and specific light conditions on the plateau, helping to protect fertilized eggs from predators while allowing embryos to develop normally in extreme environments. It can be seen that the color of fertilized eggs of fish, as a part of evolution, reflects the adaptability of species to the environment, and this evolutionary trend reflects the adaptability of the evolutionary characteristics of fertilized eggs of cypriniformes fishes in different ecological niches, thus ensuring the continuity of species in the extreme environment of the plateau.

Changes in the fish's living environment and food composition lead to changes in egg color.[29] The color of fertilized eggs of *G. dybowskii* from Manas River (faint yellow), Kaidu River (orange or light yellow)[14] and Kunes River (bright yellow)[15,16] was compared, and it was found that the color of eggs produced by the parents of three different geographical populations was different. Wild *G. dybowskii* captured in Kaidu River was directly artificially inseminated and observed, while the parent fish were transferred to the breeding station for domestication and artificial oxytocin in Kunes River. Related scholars[30-33] also found in the study of embryonic development of *S. biddulphi* and *Aspiorhynchus laticeps*, that the change of egg color was affected by living environment and food.

SIMILARITIES AND DIFFERENCES IN EMBRYONIC DEVELOPMENT WITH OTHER SPECIES

Fish embryo development is not only an important research topic in animal genetics and developmental biology, but also a key link in aquatic breeding.[34] The embryonic development of *G. dybowskii* went through the stages of blastoderm formation, cleavage, blastula, gastrula, neurula embryo, organ formation and membrane hatching, which followed the law of embryonic development of freshwater bony fish.[35] During the development of *G. dybowskii*, the muscular effect period appeared before the lens appearance period, which was opposite to that of *S. irregularis*,[36] *S. eurystomus*,[37] and *S. dolichonema*.[38] Moreover, in the late stage of embryonic development, the membrane emerges first from the tail of *G. dybowskii*, which is similar to *D. maculates*[39] and *S. pseudaksaiensis*.[23] However, *S. prenanti*[15] and *S. davidi*[25] did not show similar characteristics in film release mode, which is related to different fish species.[40]

EFFECT OF WATER TEMPERATURE ON EMBRYONIC DEVELOPMENT

Temperature is one of the key factors affecting fish reproduction. Too low water temperature prolongates the incubation period, which affects the hatching rate, and too high water temperature increases the malformation rate.[41-44] Compared with the Manas River, Kaidu River[14] and Kunes River,[15,16] the development time of *G. dybowskii* was similar in Manas River and Kunes River, while the development time of *G. dybowskii* was longer (240 h 49 min) in Kaidu River. The key stages and developmental characteristics of the blastoderm of the three geographic populations are similar, but the developmental duration and accumulated temperature are different, which may be caused by different hatching water temperatures. The artificial incubation water temperature of *G. dybowskii* in Kunes River was high, which was always maintained at 12-15℃, lower than the incubation water temperature in this study, while the lowest incubation water temperature of *G. dybowskii* in Kaidu River was 9-13℃, resulting in differences in the embryonic development time and development stage at membrane emergence of different geographical populations.

Schizothoracinae is a cold-water fish inhabiting high altitude and low temperature water environment, and its breeding water temperature is relatively low.[@350835;[36, 37,39,45] Compared with the embryonic development of other Schizothoracinae, such as *A. laticeps*,[31] *Diptychus maculates*,[39] *S. biddulphi*,[45] *S. irregularis*,[36] and *S. eurystomus*,[37] it can be seen that the incubation temperature of the embryos does not exceed 22 ℃. This is because their habitat determines their reproduction and development and the water temperature is relatively low. Low temperature can delay embryonic development of fish eggs, reduce energy consumption, effectively resist strong changes in external temperature, and thus improve the survival rate of embryos.[46]

In this study, there was no significantly different between the embryonic development water temperature of *G. dybowskii* (16.23±0.52℃) and that of *A. laticeps* (16.27±0.41 ℃),[29] but it was lower than that required for the development of *S. biddulphi* (19 ~ 19.2 ℃) and *S. pseudaksaiensis* (19 ~ 21 ℃).[23,45] This is because *G. dybowskii* mainly distributed in the three tributaries of the upper reaches of the Ili River, and *S. pseudaksaiensis* lived in the middle and lower reaches of the basin.[47] Although *S. biddulphi* is located in the middle and upper reaches of the Tarim River basin,[48] this may be due to the fact that the average water temperature in the Tarim River basin is higher than that in the Ili River.[1,49-51] It can be seen that the characteristic differences of fish embryo development are closely related to the ecological environment of the water they inhabit, and these characteristics are also a manifestation of their adaptation to the ecological environment of the water.

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AUTHORS' CONTRIBUTION

Conceptualization: Chengxin Wang; Methodology: Jiangling Li, Linghui Hu; Formal analysis and investigation: Yong Song, Jiangling Li; Writing - original draft preparation: Chengxin Wang; Supervision: Yong Song, Gulden Serekbol; Writing - review and editing: Shengao Chen.

COMPETING OF INTEREST – COPE

No competing interests were disclosed.

ETHICAL CONDUCT APPROVAL – IACUC

All experimental protocols were approved by the Science and Technology Ethics Committee of Tarim University (approval code: TDD-KYXF 20200426, approval date: 26 April 2020) and adhered to animal welfare laws, guidelines and policies.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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REFERENCES

1. Ren M, Guo Y, Zhang Q, et al. *Fisheries Resources and Fishery Ofriver Yili*. Heilongjiang Science and Technology Press; 1998.

2. Guo Y, Zhang R, Cai L. *Xinjiang of Fishery*. Xinjiang Science and Technology Press; 2012.

3. Wu Y, Wu C. *The Fishes of the Qinghai-Xizang Plateau*. Sichuan Science and Technology Press; 1992.

4. Notice on the Release of the List of Wildlife under Key Protection in Xinjiang Uygur Autonomous Region (Revised). Accessed September 21, 2022. https://www.xinjiang.gov.cn/xinjiang/c112543/202209/aaaf1dd3516e46d99086b7e378c8243b.shtml?cnName=%C3%A6%E2%80%9D%C2%BF%C3%A5%C2%BA%C5%93%C3%A4%C2%BB%C2%A4l

5. Zhao X, Mi B, Liang C, Ma Y, Sun B, Chang Y. Genetic Diversity Analysis of Gymnodiptychus dybowskii and Diptychus maculates. *Chinese Journal of Fisheries*. 2019;32(02):19-24.

6. Guo Y, Hu W, Mo C, et al. An analysis of genetic diversity in Gymnodiptychus dybowskii based on mtDNA cytochrome b gene sequences. *Journal of Dalian Ocean University*. 2016;31(05):528-532. doi:10.16535/j.cnki.dlhyxb.2016.05.010

7. Niu Y. *Study on the Individual Biology of Gymnodiptychus Dybowskii Kessler in the Ili River*. Tarim University; 2015.

8. Niu J, Cai L, Li H, Ma X. Spatial Distribution, Gonad Development and Reproductive Isolation Mechanism in Two Schizothoracins in Yili River. *Chinese Journal of Fisheries*. 2015;28(04):1-5. doi:10.3969/j.issn.1005-3832.2015.04.001

9. Zhang Y, Chen S, Wang C, et al. Morphological Differentiation of Gymnodiptychus dybowskii Kessler among Different Geographical Populations. *Xinjiang Agricultural Sciences*. 2023;60(02):511-520. doi:10.6048/j.issn.1001-4330.2023.02.031

10. Meng W. *Study on the Phylogeographic Pattern of Two Specialized Grades Schizothoracine Fishes*. Xinjiang University; 2019.

11. Niu Y, Ren D, Chen S, Cai L, Niu J, Xie C. Growth Characteristics of Gymnodiptychus dybowskii Kessler in Three Tributaries of the Ili River in Xinjiang, China. *Journal of Hydroecology*. 2015;36(06):59-65. doi:10.15928/j.1674-3075.2015.06.009

12. Haysa A, Guo Y, Meng W, Yang T, Ma Y. Phylogeny and divergence time estimation of Schizothoracinae fishes in Xinjiang. *Hereditas*. 2014;36(10):1013-1020. doi:10.3724/SP.J.1005.2014.1013

13. Yang T, Meng W, Hai S, Zhang R, Guo Y. Molecular Phylogeny of Schizothoracinae Fishes in Xinjiang Based on Mitochondrial Cytochrome b Gene Sequences. *Arid Zone Research*. 2011;28(03):555-561. doi:10.13866/j.azr.2011.03.012

14. Liu M, Tan G, He X, et al. Observation on embryonic development of Gymnodiptychus dybowskii in Kaidu River Basin. *Jiangsu agricultural sciences*. 2020;48(03):175-179. doi:10.15889/j.issn.1002-1302.2020.03.030

15. Zhang Q, Bilal E, Li S, Bribrihan, Huang S, Eliam T. Study on embryo development and hatching techniques of Gymnodiptychus dybowskii. *Xinjiang agricultural reclamation technology*. 2019;42(10):31-33.

16. Niu J, Zhang T, Liu H, Minawar M, Cai L, Liu C. Morphological Observation of Embryonic and Early Larval Development of Gymnodiptychus dybowskii. *Chinese Journal of Fisheries*. 2019;32(01):1-6.

17. Wang D. The Schizothoracinae Fishs in Xinjiang. *Arid Zone Research*. 1998;15(4):26-32. doi:10.13866/j.azr.1998.04.006

18. Wang W. *Augmentation of Fish Culture*. China Agriculture Press; 2000.

19. Zhang Y, Wang T, Jiao W, Du Y, Shi X. Morphology Observation of the Embryonic Development of Gymnodiptychus pachycheilus. *Sichuan Journal of Zoology*. 2013;32(3):389-392. doi:10.3969/j.issn.1000-7083.2013.03.014

20. Yin M. Study on early life history of fishes and its progress. *Journal of Aquatic Sciences*. 1991;15(4):348-358.

21. Yin M. *Fish Ecology*. China Agriculture Press; 1995:136-137.

22. Wu X. *Annals of Cyprinid Fishes of China*. Shanghai Science and Technology Press; 1964.

23. Cai L, Niu J, Zhang B, et al. Observation on embryonic and larval development of Schizothorax pseudaksaiensis Herzenstein. *Freshwater Fisheries*. 2011;41(5):74-79. doi:10.3969/j.issn.1000-6907.2011.05.013

24. Zuo P, Li G, Leng Y, et al. Embryonic and Early Larval Development of Schizothorax wangchiachii. *Journal of Hydroecology*. 2015;36(3):77-82. doi:10.15928/j.1674-3075.2015.03.013

25. Hu R, Zhang Y, Shu Q, et al. Observation on embryonic development of Schizothorax davidi. *Jiangsu agricultural sciences*. 2020;48(14):198-203. doi:10.15889/j.issn.1002-1302.2020.14.037

26. Chen S, Song Y, Niu Y, et al. Embryonic and post-embryonic development of Triplophysa (Hedinichthys) yarkandensis (Day). *Journal of Fishery Sciences of China*. 2015;22(4):597-607. doi:10.3724/SP.J.1118.2015.150012

27. Wang H, Guo Y, Zuo L, Mi J, Xiong Y, A W. Embryonic and Larval Development of Triplophysa angeli. *Fisheries Science*. 2009;28(12):721-725. doi:10.3969/j.issn.1003-1111.2009.12.003

28. Li Z, Yan T. Morphological Development of Triplophysa Bleekeri (Sauvage et Dabry de Thiersant, 1874) Embryo and Larvae. *Acta Hydrobologica Snica*. 2009;33(4):636-642. doi:10.3724/SP.J.0000.2009.40636

29. Han J, He J, Chen P, et al. Observation on embryonic development, morphology and growth of larvae and juveniles of Aspiorhynchus laticeps. *South China Fisheries Science*. 2021;17(01):59-66. doi:10.12131/20200177

30. Xie C, Zhang R, Ma Y, Tuersun, Guo Y, Xu G. Preliminary Study on Artificial Propagation of Schizothorax biddulphi Günther. *Arid zone Research*. 2010;27(5):734-737. doi:10.13866/j.azr.2010.05.028

31. Ren B, Ren M, Guo Y, et al. Studies on The Biological characteristics of Aspiorhynchus laticeps (Day). *Chinese Journal of Fisheries*. 2007;19(2):9-22. doi:10.3969/j.issn.1005-3832.2006.02.002

32. Ren B, Ren M, Guo Y, et al. Morphological observation of the development of Aspiorhynchus laticeps embryo and larva. *Journal of Dalian Ocean University*. 2007;22(6):397-402. doi:10.16535/j.cnki.dlhyxb.2007.06.007

33. Zhang R, Ma Y, Tuersun, et al. Preliminary Observation on Embryonic and Fry Upgrowth of Aspiorhynchus laticeps (Day). *Arid zone Research*. 2008;25(2):190-195. doi:10.13866/j.azr.2008.02.024

34. Bobe J, Labbé C. Egg and sperm quality in fish. *General and Comparative Endocrinology*. 2010;165(3):535-548. doi:10.1016/j.ygcen.2009.02.011

35. DeMartini E. Annual variations in fecundity, egg size, and the gonadal and somatic conditions of queenfish Seriphus politus (Sciaenidae). *Fishery Bulletin*. 1991;89(1):9-18.

36. Zhao H, Hong J, Zhao N, Hu R, Wei J, Niu Z. Study on the artificial propagation technology,embryonic and early larval development of Schizothorax irregularis in the Kizil River in Xinjiang. *Journal of Dalian Ocean University*. 2021;36(03):414-422. doi:10.16535/j.cnki.dlhyxb.2020-201

37. Xia Z, Zhao H, Qiu M, et al. Artificial propagation, embryonic and early larval development of Schizothorax eurystomus. *Journal of Dalian Ocean University*. 2022;37(03):471-481. doi:10.16535/j.cnki.dlhyxb.2021-132

38. Xu Y. Observation of Egg and Embryonic Development of Schizothorax dolichonema Herzenstein by Artificial Breeding and Propagation Technique. *Southwest China Journal of Agricultural Sciences*. 2019;32(04):942-946. doi:10.16213/j.cnki.scjas.2019.4.038

39. Wang C, Chen S, Wang X, Zi F, Lin X, Wei Q. Observation on Embryonic Development and Post-embryonic Development of Distychus maculates in Turks River, Xinjiang. *Chinese Journal of Zoology*. 2022;57(05):668-677. doi:10.13859/j.cjz.202205003

40. Xu B, Wei K, Zhu X, et al. Embryonic and Larval Development of Gymnocypris potanini firmispinatus in Yalong River. *Journal of Hydroecology*. 2018;39(5):67-75. doi:10.15928/j.1674-3075.2018.05.010

41. Orton J. Sea temperature, breeding and distribution in marine animals. *Journal of the Marine Biological Association of the United Kingdom*. 1920;83:133-150. doi:10.1017/S0025315400000102

42. Xue L. Effects of exogenous hormone, temperature and parental weight on artificial induced spawning and incubation of Mastacembelue armatus. *Acta Hydrobiologica Sinica*. 2018;42(2):333-341. doi:10.7541/2018.042

43. Qi Z, Zhang X, Han S, Jiang Y, Tian Y. The effect of temperature on embryonic development of Esox lucius. *Freshwater Fisheries*. 2010;40(4):76-79. doi:10.3969/j.issn.1000-6907.2010.04.014

44. Chen F, Hu J, Wang Y, Chen J. The effect of temperature on embryonic development of Puntius conchonius. *Freshwater Fisheries*. 2013;43(1):24-27. doi:10.3969/j.issn.1000-6907.2013.01.005

45. Zhao N, Xu X, Zhao H, et al. Artificial Propagation Technology, Embryonic and Early Larval Development of Schizothorax biddulphi. *Acta Agriculturae Boreali-occidentalis Sinica*. 2021;30(12):1767-1776. doi:10.7606/j.issn.1004-1389.2021.12.002

46. Gan X. *Effects of Temperature on Reproductivity, Embryonic Development and Growth of Gobicypris Rarus*. Southwest University; 2012. doi:10.7666/d.y2086029

47. Cai L, Niu J, Liu C, et al. Species Diversity and Dominant Fish Species in Different Reaches of the ili river, Xinjiang. *Acta Hydrobiologica Sinica*. 2017;41(04):819-826. doi:10.12131/20200177

48. Ma Y, Guo Y, Zhang R, et al. Fauna composition and distribution of aboriginal fish in the Tarim River of Xinjiang Uygur Autonomous Region. *Journal of Fisheries of China*. 2009;33(06):949-956. doi:10.3724/SP.J.00001

49. Tang J, Zhang J, Ren D. Analysis of water quality in Tarim River Basin. *Water conservancy fishery*. 2005;25(02):67-68. doi:10.15928/j.1674-3075.2005.02.028

50. Chen S, Chen Y, Fan Z, Niu Y, Xie C, Lei H. Spatial-Temporal Characteristics of Water Quality in the Upper Reaches of the Tarim River in Alar. *Journal of Hydroecology*. 2014;35(05):15-21. doi:10.15928/j.1674-3075.2014.05.007

51. Li X, Ni C, Peng L, et al. Phytoplankton Community Structure and Its Relationship with Environmental Factors in Yili River of Xinjiang Uygur Autonomous Region. *Journal of Hydroecology*. 2022;43(06):59-68. doi:10.15928/j.1674-3075.202105070136