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Incubation and Larval Development Durations of Sterlet (*A. ruthenus* LINNAEUS 1758) in River Water Rearing under Near-Natural Conditions

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Abstract: Day-degrees [°D], also known as accumulated thermal units (ATUs), have been used to predict the duration of early development for fish. For most sturgeon species, the available data in the literature were determined under temperature-constant conditions. However, there is a lack of information on ATUs of early development for sturgeons under natural or near-natural temperature conditions. The aim of this study was to observe the duration of incubation and the duration from hatch until feeding of sterlets (*Acipenser ruthenus*) under near-natural conditions. This study was embedded within the LIFE-Sterlet and LIFE-Boat 4 Sturgeon project and data were gathered from the year 2017 to 2023. The rearing of fish larvae took place in the project hatchery container with Danube water without biological, chemical, or thermal water treatment to simulate natural conditions. Temperatures [°C] were monitored on a daily basis and day-degrees were calculated by summing the temperature over time. Results indicated slower larval development than described in the literature. Hatching started earliest after 7 days at 106 °D and latest after 151 °D. Feeding started between 155 and 271 °D. The findings of this study provide valuable insights into the temperature-dependent development of sterlet larvae under near-natural conditions and can assist in the design of optimal rearing of sturgeons for conservation efforts.

Keywords: sterlet; larval development; incubation duration; river rearing

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1. Introduction

Aquatic ecosystems are facing numerous challenges due to human alterations and activities [1,2]. One specific group of animals that is significantly impacted are long-distance migratory species such as sturgeons, which are nowadays the most threatened group of animals worldwide [3]. Their decline was mainly driven by overexploitation, migration barriers, habitat loss, pollution, and hybridization [4–6].

In the Danube River Basin, two of the historically six species are already extinct and the remaining four species are classified as endangered and critically endangered [7]. In the Lower Danube, the construction of the hydropower plants (HPP) Đerap I and II blocked migration routes and led to a loss of important upstream spawning grounds [8,9]. As a consequence, all remaining anadromous species are locally extinct in the Upper and Middle Danube [9,10], and the sterlet (*Acipenser ruthenus*) is the only sturgeon species that is still present [11]. In the Upper Danube, the total reproductive population is estimated to be less than 1000 individuals [10], and the only self-sustaining population at the German–Austrian border has a reproductive population size of 60–99 individuals [12]. One remnant population was discovered in 2014 during net monitoring below the HPP Freudenau downstream of Vienna with an estimated maximum population size of 80 individuals [13]. However, this population presumably has lost its spawning grounds after the construction of the HPP Freudenau [11], and no natural reproduction has been recorded since 1986 [12,13].

The Pan-European Action Plan for sturgeons [14] states that in addition to in situ conservation actions, ex situ measures, in particular, are inevitably necessary for the safeguarding, recovery, and re-establishment of highly threatened sturgeon species.

From 2016 to 2022, the project LIFE-Sterlet aimed to restore the sterlet population in the Upper Danube. The focus of the project was to raise sterlet juveniles under near-natural conditions with emphasis on training for survival and adaption to wild conditions. Therefore, the conservation hatchery was placed on the Danube Island (Vienna) at a 50 m distance from the Danube. Water was pumped from the Danube into the hatchery and was, besides a drum filter with a 50 µm mesh and UV treatment, chemically and thermically untreated. This allowed the sterlets to grow up under a natural temperature regime, turbidity, and fine sediment load. The use of river water further promotes imprinting to the home water body and, as a result, induces homing behavior. Over the whole project time, about 200,000 individuals were released into the Danube, Morava, and Thaya Rivers. The efforts were continued and extended within the LIFE-Boat4Sturgeon Project (2022–2030), including now all remaining Danube sturgeon species.

Almost all available information about the early-life-phase duration of sturgeons comes from commercial aquaculture under controlled and stable conditions. Due to the lack of information on accumulated thermal units (ATUs), also known as Day-degrees [°D], of early development in sturgeons under natural or near-natural conditions, this study aims to give first insights into the embryonal and larval development durations of sterlet, reared under near-natural conditions with untreated river water.

2. Materials and Methods

2.1. Gamete Collection and Incubation

Sexually mature sterlets were caught in Spring 2018 to 2023 via net fishing in the Austrian Danube downstream of Vienna and transported to the LIFE-Sterlet hatchery on the Danube Island. As the whole hatchery system is running with thermically untreated Danube water, no acclimatization time was needed and the individuals were transferred into tanks outdoors.

Upon determination of the polarization index of ovarian follicles, spawning was induced hormonally, using CCP (acetone-dried common carp pituitary) [15–17]. The eggs were stripped directly into a clean and dry bowl and, after removal of the ovarian fluid, were mixed with fertilizing solution (sperm/water ratio 1:200) to avoid polyspermy. The embryos were stirred carefully until the eggs became adhesive. The de-adhesion of eggs was performed with whole milk (2017–2022) and talcum (2023) for 60 min, gently stirring the bowls. Females were stripped every two hours to collect ripe eggs in the oviduct. The procedure was repeated five times per female. Sperm was collected from the males using a syringe and catheter for every individual spawning process immediately after the female stripping. Egg batches were individually fertilized by the sperm of two males and then pooled for incubation in 2019, 2021, and 2023. In 2020 and 2022, the eggs of individual females were fertilized with the sperm of individual males.

2.2. Embryos from Other Sources

From 2017 to 2019, a share of the embryos was sourced from a fish farm in Germany. Additionally, from 2018 to 2021, embryos were sourced from wild fish in Hungary, which were incubated for four days at a constant 16 °C at a project partner within the DTP-Measures project.

2.3. From Egg Incubation until Hatching

After the de-adhesion, the embryos were transferred into McDonald jars (volume 6.5 L), which were connected via hoses with the water supply pipes. The water supply was kept at 2–2.5 L/min, which corresponds to 30–40% of the jar volume per minute [15]. The water was pumped from the Danube through a drum filter, and then treated with a UV (ultraviolet) light sterilization system to prevent saprolegniosis. The temperature was measured at a sampling interval of 24 h every day at noon and the calculation of the day-degrees continued until the end of hatching, using the following equation:

$$\text{Day-degrees} = \sum_{i=1}^{n} T_i$$

where T_i is the temperature measured on day i, and n is the total number of days for which temperature measurements are available. One thermal unit (one day-degree) therefore accumulates when held in water of 1 °C for 24 h and is added for every additional 24 h period.

2.4. From Hatching until Feeding

The beginning of the hatching also marked the start of a new day-degree counting, which ended with the start of exogenous feeding. A clear sign of the transition to exogenous feeding is the loss of the melanin plug from the anal opening, and feeding should start once 2–3% of the larvae lost their melanin plug [15]. A light source placed at the middle and at the upstream end of the basins helped to keep the larvae away from the net at the tank outlets, concentrating the positive photoactive larvae at the illumination sources.

2.5. Daily Temperature Fluctuations

Daily temperature fluctuations were not measured in the hatchery, as the measurements were taken only once per day. The closest gauging station (Wien-Nussdorf) is about 5 km upstream from the hatchery and measures the temperature continuously at an interval of one hour (Table S1, data provided by viadonau). The average daily temperature fluctuations for the different periods were calculated for all families. The fluctuations were between 0.2 °C and 1.6 °C (Tables S2 and S3). It can be assumed that the temperature fluctuations measured at the gauging station correspond to the temperature regime 5 km further downstream.

3. Results

3.1. From Incubation until Hatch

Hatching started earliest after 7 days at 106 °D, the lowest day-degrees were 103 °D after 9 days, with 117 ± 17 °D on average (Table 1), and the latest with 151 °D. It ended with the latest after 217 °D, lasting between 3 and 9 days.

3.2. From Hatch until Feeding

Feeding started earliest after 155 °D averaging at 186 ± 20 °D (Table 1). The longest duration from hatching until feeding was in the year 2017 with 271 °D, taking 24 days.

Table 1. Development durations in day-degrees [°D] and days for hatching and feeding of the cohorts from Austria (AT), Hungary (HU), and Germany (DE). * Average hatch duration temperature for Hungarian batches was calculated using only measurements from the project hatchery. Cohorts from the AT wild population are highlighted in bold.

ID	Year	Origin	Arrival Danube Water Incubation [°D]	Average Temperature Start Hatch [°C]	±SD	Min [°C]	Max [°C]	Start Hatch [°D]	Start Hatch [Days]	Hatch Duration [Days]	Average Temperature Start Feeding [°C]	±SD	Min [°C]	Max [°C]	Start Feeding [°D]	Start Feeding [Days]
1	2017	DE	-	-	-	-	-	-	-	-	11.3	0.89	10	12.9	271	24
2	2018	HU	64	13.2 *	0.2	13.0	13.7	130	-	-	14.6	0.9	13.1	16.0	174	12
3	**2018**	**AT**	-	**14.5**	**0.6**	**14.0**	**16.0**	**120**	**8**	**3**	**15.6**	**0.5**	**14.6**	**16.2**	**171**	**11**
4	**2018**	**AT**	-	**15.2**	**0.4**	**14.6**	**16.0**	**106**	**7**	**3**	**15.7**	**0.5**	**14.6**	**16.2**	**173**	**11**
5	2019	HU	52	10.2 *	0.4	9.8	10.8	104	-	-	11.7	0.6	10.4	12.7	187	16
6	**2019**	**AT**	-	**11.4**	**0.7**	**10.0**	**12.2**	**103**	**9**	**9**	**11.3**	**0.7**	**10.2**	**12.7**	**204**	**18**
7	**2019**	**AT**	-	**10.8**	**0.4**	**10.2**	**11.3**	**108**	**10**	**6**	**12.2**	**1.0**	**10.4**	**13.7**	**159**	**13**
8	2019	DE	-	-	-	-	-	-	-	-	11.2	0.7	10.3	12.5	169	15
9	2020	HU	60	12.1 *	0.4	11.8	12.8	109	-	-	13.2	0.6	11.8	14.2	212	16
10	**2020**	**AT**	-	**13.4**	**0.3**	**13.0**	**14.0**	**107**	**8**	**6**	**14.2**	**0.4**	**13.5**	**14.8**	**212**	**15**
11	**2020**	**AT**	-	**14.5**	**0.5**	**13.8**	**15.3**	**145**	**10**	**6**	**15.9**	**0.5**	**14.2**	**15.8**	**194**	**13**
12	2021	HU	64	9.7 *	0.5	8.7	10.6	151	-	-	12.1	1.6	9.4	14.3	218	18
13	**2021**	**AT**	-	**11.8**	**1.3**	**10.3**	**14.2**	**118**	**10**	**6**	**14**	**0.4**	**13.2**	**14.7**	**209**	**15**
14	**2022**	**AT**	-	**12**	**0.5**	**11.3**	**12.7**	**108**	**9**	**5**	**14.4**	**0.9**	**12.7**	**15.6**	**173**	**12**
15	2023	HU	68	9.2 *	0.9	8.2	11.2	151	-	-	11.9	1.0	10.5	13.8	191	12
16	**2023**	**AT**	-	**12.2**	**1.0**	**10.5**	**13.1**	**110**	**9**	**9**	**12.9**	**0.6**	**11.9**	**14.0**	**155**	**16**

4. Discussion

4.1. From Incubation until Hatch

The duration of early development in fish strongly depends on the water temperature [15,18]. In the literature, different information for sterlets on incubation durations is given (Table 2). In hatchery facilities with the ability to control the water conditions, the temperature should be kept within the optimal species-specific temperature range, close to the average value for incubation [15]. The optimal temperature for sterlets is, according to Chebanov and Galich [15], between 13 °C and 16 °C. The authors further state that the incubation period lasts 6-11 days Hochleithner and Gessner [17] and Holčík [18] state that at 13 °C it lasts 4–5 days (52–65 °D). The observations of this study showed that the fastest incubation period took 7 days (106 °D) (Table 1). The measured temperature at this period was between 14.6 and 16.0 °C, averaging at 15.2 ± 0.4 °C, and was therefore within the recommended optimal temperature limits and expected duration (6–11 days) mentioned in Chebanov and Galich [15] and Gela et al. [19]. The same applies to the batches ID 3, 10, and 11, where the temperature within this period ranged between 13.0 °C and 16.0 °C, and the development duration until hatch took 8 to 10 days. However, most of the literature proposes a duration from 4 to 7 days at 13 °C, and Manea [20] suggests a duration from 6 to 7.4 days at temperatures between 13 °C and 16 °C, showing shorter development durations at similar temperatures. This comparison also applies to the batches ID 6, 7, 13, 14, and 16 (temperature range: 10.0–14.2 °C), where the incubation took 9–10 days, which is longer than in most literature findings. On the other hand, Igumnova [21] stated that the development until hatching of free embryos of sterlet from the Volga River takes more than 13.3 days at 10 °C, indicating that under natural conditions, development might take longer than described in aquaculture.

Table 2. Incubation durations of sterlet in the literature.

Reference	Temperature [°C]	Degree Hour [°h]	Day-Degrees [°D]	Days
Igmnova [21]	10		133	>13.3
Shmidtov [22]	10–11		90–77	9–7
Shmidtov [22]	13		52–65	4–5
Manea [20]	13	2140	89	6.8
Hochleithner and Gessner [17]	13		52–65	4–5
Manea [20]	13–16	2300	96	7.4–6
Gela et al. [19]	15		90	6
Manea [20]	16–18	1920	80	5–4.4
Gela et al. [19]	17.8		89	5
Igmunova [21]	20		67	<3.3

Duration varied also from hatch-begin until hatch-end between 3 to 9 days. According to Dettlaff et al. [16], the duration of the hatching period can vary significantly in different batches of embryos and in the same batch at different levels of incubator loading. Possible explanations for the slower development and also the higher level of variation in the project hatchery are turbidity, potential pollution, or bacterial and fungal load, especially at high water events. Furthermore, slight temperature fluctuations during the day might have resulted in prolonged development duration.

The study's limitations include a small sample size, suggesting that obtaining more precise results would necessitate larger data sets for a thorough understanding of the correlation between temperature and development duration under near-natural conditions. However, almost all calculated ATUs were higher than described in the literature for the respective temperature. Looking at the duration from embryo incubation until hatch in days, the literature presents considerable variability in the reported data, predominantly indicating shorter developmental timescales than observed in this study.

4.2. From Hatch until Feeding

Exogenous feeding started earliest after 11 days at 171 day-degrees, the lowest day-degrees were at 155 °D after 16 days, averaging at 186 ± 20 °D (Table 1). The longest duration from hatching until feeding was in the year 2017 (ID 1, Table 1) with 271 day-degrees at an average temperature of 11.3 ± 0.89 °C (temperature range 10.0–12.9 °C), taking 24 days. In comparison to the cohort ID 6 in 2019, which had the same average temperature of 11.3 ± 0.7 °C and a similar temperature range (10.2–12.7 °C), more days with temperatures below 11 °C were documented in 2017 in ID 1 (Table S4). Therefore, the longer duration of this period for the 2017 cohort could be due to the lower temperatures occurring during the incubation period. The range of the daily temperature fluctuations for both periods exhibits a close resemblance (Table S3).

In general, active food intake starts 150–220 °D after hatch [15,17], and the period from hatching to the beginning of exogenous feeding is, according to Dettlaff et al. [16], at least as long as the embryonic development. This would suggest that, despite the described outlier (ID 1), all findings from this study are in line with the information found in the literature.

4.3. Conservational Aspects

To prevent the remaining sturgeon species in the Danube River Basin from extinction, in situ and ex situ measures are of the utmost importance. Ex situ actions are, in consideration of the morphological situation and the lack of migration corridors and longitudinal connectivity for the remnant wild populations, especially necessary to keep the genetic diversity of the four Danube sturgeon species [14]. Artificial propagation methods of sturgeons are globally established. Despite the existing hatchery guidelines and knowledge, there are still knowledge gaps about the successful breeding, rearing, and management of exploited populations [23]. The study's results present valuable insights into near-natural sturgeon rearing and contribute to closing the existing knowledge gap.

For the remnant population below Vienna, no reproduction has been recorded for more than 40 years [12,13], and within the LIFE-Sterlet project, several females were captured with eggs in resorption. This indicates that in this Danube stretch, no suitable spawning grounds are available or that there is a lack of suitable spawning mates. Therefore, the gathered data in this study provide the first and, so far, the only available insights into the development durations for sterlets associated with the wild Viennese sterlet population. Knowledge of development durations is not only valuable for rearing purposes but can also help to plan restoration measures and monitoring actions. An approximate estimation of the start of the exogenous feeding, for example, can assist in planning restoration measures, as from this stage onwards, the larvae start to drift freely in the water column. Furthermore, the timing and placement of larval drift nets for monitoring purposes can be optimized.

However, the concept of ATUs should be regarded with caution. The relation between temperature and development duration is not strictly linear [16,24]. Calculated predictions on this basis are therefore only approximations [24]. An alternative to ATUs would be a development index, which already exists for other sturgeon species like lake sturgeon (*Acipenser fulvescens*) [25]. Such indices accurately predict the time of larval development stages (e.g., neural tube closure, hatch, exogenous feeding) in percentages of the total duration of embryogenesis at any given temperature [25]. The duration of different developmental periods, expressed in fractions of the total duration of embryogenesis, remains constant at different optimal temperatures for, e.g., Russian and stellate sturgeon (*Acipenser gueldenstaedti, Acipenser stellatus*) [16], which would suggest that such indices would provide a more stable and reliable prediction tool than ATUs. However, the development of the index mentioned in Eckes et al. [25] required the euthanization of eggs and larvae for each important development stage at four different temperatures and four respective replicates. The implementation of an index would not have been possible within the conservation hatchery due to the small egg quantities. Considering the circumstances and the conservational aspects of the two LIFE projects, the utilization of the ATUs instead of a development index was preferable. However, the information on ATUs provided by this study can become more precise over time by including a longer time series of the data. The LIFE-Boat4Sturgeon project is to be continued until the year 2030 and will therefore not only provide further data on sterlet rearing but also gather information on the rearing of all other Danube sturgeon species.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ecologies5020016/s1, Figure S1: Duration from hatch until feeding in day-degrees [°D] in the year 2017; Figure S2: Duration from egg incubation until hatch in day-degrees [°D] in the year 2018; Figure S3: Duration from hatch until feeding in day-degrees [°D] in the year 2018; Figure S4: Duration from egg incubation until hatch in day-degrees [°D] in the year 2019; Figure S5: Duration from hatch until feeding in day-degrees [°D] in the year 2019; Figure S6: Duration from egg incubation until hatch in day-degrees [°D] in the year 2020; Figure S7: Duration from hatch until feeding in day-degrees [°D] in the year 2020; Figure S8: Duration from egg incubation until hatch in day-degrees [°D] in the year 2021; Figure S9: Duration from hatch until feeding in day-degrees [°D] in the year 2021; Figure S10: Duration from egg incubation until hatch in day-degrees [°D] in the year 2022; Figure S11: Duration from hatch until feeding in day-degrees [°D] in the year 2022; Figure S12: Duration from egg incubation until hatch in day-degrees [°D] in the year 2023; Figure S13: Duration from hatch until feeding in day-degrees [°D] in the year 2023; Table S1: Temperature measurements for the months April and May from 2017 to 2023 from the gauging station Wien-Nussdorf measured at an one hour interval. The data was provided by viadonau.; Table S2: Daily temperature range from the gauging station Wien-Nussdorf in the years 2017 to 2023 for the period from incubation until hatching for each family; Table S3: Daily temperature range from the gauging station Wien-Nussdorf in the years 2017 to 2023 for the period from hatching until feeding for each family; Table S4: Temperature values measured at the hatchery.

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