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DEVELOPING THE BIOTECH METHOD FOR EFFECTIVE REPRODUCTION OF VALUABLE FISH SPECIES POPULATIONS

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Abstract

Artificial reproduction of sturgeons and salmonids has shortcomings resulting in a return of commercially raised breeders. These disadvantages primarily are the catch of Atlantic salmon producers in spawning beds, which causes detriment of natural reproduction, and low survival rate of farmed juveniles in nature. In order to increase the efficiency of commercial fish reproduction, we suggest the new methods which are based on fish-specific adaptations during marine feeding period, providing the greatest population productivity and survival. Our objective was the development of a biotechnology for effective farm reproduction of salmon *Salmo salar* (Linne, 1758) and sturgeon *Acipenser stellatus* (Pallas, 1771). First, three main effects have been experimentally established for commercial fish culture in brackish seawater close to critical salinity (4-8 ‰): the highest survival rate, prolongation of high reproductive quality of breeders, and acceleration of juvenile development and growth. Physio-biochemical analysis showed the minimal losses of hemoglobin and serum protein in critical salinity medium, with maximum retention of salts in the blood and in ovarian fluids, apparently by optimizing water-salt balance of the body. The latter is achieved through optimal osmotic gradient between the inner and outer media close to critical salinity limits values. This energy-saving gradient ensures water-salt metabolism and homeostasis of the internal medium and thus the external medium (critical salinity) provides bio-stimulating effect that increases the body resistance. The maintenance of fish brood stocks in the critical range of seawater salinity (3.07-8 ‰) until puberty of producers is proposed by us as an effective method of reproducing populations of Sevruga and Baltic salmon. Then, the breeders naturally matured under specific range of the seawater salinity below a 3.06 ‰ threshold are used to obtain mature sex products. Fertilized eggs are incubated in fish-farm in river water where then larvae and juveniles grow. When recruits sign of readiness to migrate they are placed in seawater salinity 2.5-7 ‰ close to critical range and grow to viable stages. Results of comparative industrial tests of the new biotech reproduction of sturgeon and salmonids in sturgeon and salmon fish farms and in marine cages have shown the effectiveness and advantages of this method which allows preservation of high breeding quality Sevruga producers up to 100 %, and a 5-7-fold growth enhancement of young salmon. This new method can help solving the common problems of rare and endangered populations of commercial fish species restoration, which is in line with a fish farming trend of Conservation Aquaculture aimed at restoring natural environment.

Keywords: fish artificial reproduction, *Acipenser stellatus*, *Salmo salar*, Baltic population, *Rutilus rutilus caspicus*, fish farming, factory sturgeon and salmonids tech breeding, fish farming in brackish sea water

The populations of Atlantic salmon in the northwestern region of Russia and sturgeon species in the southern regions of Russia have lost their commercial value, and are maintained primarily on account of artificial farm reproduction. Unlike sturgeon hatcheries isolated from spawning beds, most salmon hatcheries are located in their aquatic areas, where they remove adult producers at the expense of natural reproduction. The fishing load of salmon hatcheries on producers during breeding season and catching of valuable fish species during prespawning migration are the reason of progressive decline of their populations [1, 2]. The second reason is the low return of farm-reared producers (up to 2%), especially Atlantic salmon, in relation to the total quantities of released young fish, which is indicative of low survival rates and the need to improve the efficiency of biological technologies of farms. According to calculations, only large two-year old smolts need to be released (40 g and more) in the amounts no less than 150 thousand individuals, which is currently not being performed [2].

The biotechnological basis to solve the current issue of deficit of adult sturgeon and salmon producers [3, 4] is their long-term reservation [2, 5]. The farm technology of breeding Atlantic salmon has become obsolete, it does not envision the stages of formation and maintenance of rearing female stocks, and does not envision release and allocation of young fish in feeding basins [6]. The domestic experience and examples of implemented programs of preserving the populations of Atlantic salmon and sturgeon species in other countries point at the need of a more efficient artificial reproduction (along with restoration of the scope of natural reproduction), and increase of survival rate and heterogeneous variety of farm-reared young fish [2, 7, 8].

In this work we for the first time demonstrate a possibility of maintaining rearing female stocks, wide-scale breeding and progressive multiple acceleration of young Atlantic salmon growth in critical salinity water in case of perennial maritime cage culture fishery. As a result, the producers of starred sturgeon, salmon and roach have been successfully reserved and efficiency of maintenance and use of their rearing female stocks has been proven.

The goal of the research is to develop a complex of biotechnological measures for improvement of artificial reproduction of valuable commercial fish species.

Technoloqies. The experiments (commenced in 1976-1983 and completed in 2010-2013) were conducted at sturgeon fisheries located in Lower Volga regions (Aleksandrovskiy, Bertyulskiy, Ikryaninskiy and Kizyanskiy) and the Don River (Rogozhkinskiy, Vzmorye), and on Nevskiy salmon fishery (salmon hatchery, Leningrad Province, Vsevolozhskoy Region, village of Ostrovki) and at the premises of maritime cage culture fish farm Alkor-Farm LLC (Vyborgskiy Regions, village of Pribylovo, Klyuchevoye fishing ground). In the study starred sturgeon *Acipenser stellatus* (Pallas, 1771), Atlantic salmon *Salmo salar* (Linne, 1758) of Baltic population (Baltic salmon) and roach *Rutilus rutilus caspicus* (Jakowlew, 1870) were involved.

The data of National Research Institute of Lake and River Fish Industry (Saint Petersburg) were used and direct measurements were taken at the cages [2] in order to characterize the primary hydrochemical parameters (salinity, pH, oxygen content, etc.) in the offshore areas of the Vyborg Bay near surface of the water and in the near bottom layer. The findings were identical and conformed to the standards (apart from temperature increase by 3-4 °C during the years of thermal anomalies).

The reservation of a valuable commercial object, the starred sturgeon (69 females in pre-spawning condition) and roach as a large-scale laboratory object (above 350 specimen of both genders in pre-spawning condition) was performed simultaneously and in identical conditions at the aforementioned fish hatcheries in concrete tanks filled with sodium chloride solutions at spawning temperatures during the timeframe required for reproduction, for starred sturgeon it was 5‰ till 1 month, for roach 3, 5 and 12‰ during 55 days. The survivability rate of these species was evaluated in sodium chloride solutions of varying concentration at upper spawning temperatures of 17.4-25.8 °C and oxygen content of

5.2-7.5 mg/l. In the control group the producers were maintained in river water with all other conditions being equal. The fish-rearing qualities of starred sturgeon females were evaluated using conventional methods [9] at fatness coefficient, degree of use in fish-rearing (% of mature female), working fecundity, roe fertilization quality (%), and semen quality (using a 5-point scale).

The levels of hematoglobulin and total protein in blood were determined for evaluation of overall physiological state of fish for 50 roach individuals. For 10 starred sturgeones the osmolarity of blood plasma, chamber (ovarian) fluid and urine was determined using standard procedures [9, 10] with varying water salinity and depending on duration of reservation.

The producers of Baltic salmon were held in river water (control group) in plastic tanks in the Nevskiy salmon fishery (88 females and 75 males in pre-spawning condition) and in sea cages (test group) in the water of the Vyborg Bay with 2.5-4 ‰salinity (44 females and 32 males in pre-spawning condition); the number of mixed-age reared young fish exceeded 1.5 thousand individuals. The average weight, body length and fatness coefficient were determined for producers and young salmon, as well as female fecundity, percentage of mature producers, semen quality and percentage of hatched larvae. The mean morphometrical parameters of two-year-olds and three-year-olds (at least 30 individuals of each age group) were determined for all young fish groups reared in sea cages and were compared with their data from Nevskiy fishery and standard parameters for the Leningrad region.

The novelty of our approach is due to a formalized comparative analysis method generally accepted in patent and invention domain.

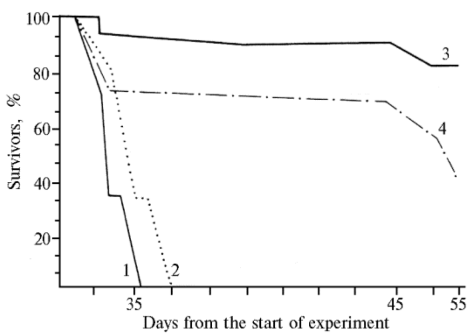

Fig. 1. The survival rate of roach (*Rutilus rutilus caspicus*) producers in sodium chloride solutions of different concentration: 1 — control group (river water), 2 — NaCl 3 ‰, 3 — NaCl 5-7 ‰, 4 — NaCl 12 ‰ (Ikryaninskiy sturgeon fishery, 1979).

The results were processed using variation statistics methods with Microsoft Excel software package. We calculated the arithmetic mean values (*M*), deviations from mean values (±SEM), mean-square deviations (σ) and variation coefficients (*Cv*, %).

Results. The deficit of adult producers of sturgeon and salmon species brings the task of creation, maintaining and operating commercial rearing female stocks to the foreground [2-4]. The developed method of lengthy commercial reservation of fish producers in brackish water with critical salinity at 4-8 ‰ [5] increases their use in fish-rearing. The critical salinity, which is the threshold salinity for maturing of gametes of marine and freshwater organisms, allows us to determine the limit of their physiological tolerance and limits of organism interactions with the environment [5, 11, 12].

We have for the first time determined the highest degree of survivability and delayed sexual maturation of roach producers and female starred sturgeons both in sea water and in industrial sodium chloride solution with the same concentration (Fig. 1, 2). In the control group (river water) and NaCl solution (3 ‰) all non-migratory individuals of roach with complete resorption of reproductive products after 35 and 38 days respectively (see Fig. 1) perished. With NaCl 5 and 12 ‰ levels the survival rate of roach producers by the end of the test constituted more than 80 and 40% respectively. The fish-rearing parameters of starred sturgeon females were analyzed in river water with 5‰ sodium chloride solution levels. The perish rate of females in the control group on the 28th day was about 20% (see Fig. 2) with mass resorption effect of reproductive cells in perished fish. For females of starred sturgeon reserved in the 5‰ solution of NaCl during the period required for reproduction (see Fig. 2) a possibility of obtaining sound progeny was observed. It was determined that critical salinity and even salinity reduced to 2.5 ‰ was optimal for maintenance of producers and reproduction of populations of anadromous species, the Baltic salmon and starred sturgeon.

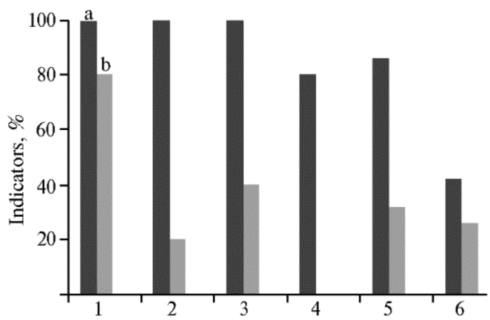

Fig. 2. Fish-rearing indicators of starred sturgeon females (*Acipenser stellatus*) in the test group (a; NaCl 5 ‰) **and in the control group** (b; river water) **28 days after reservation with upper values of spawning temperatures 17.4-25.8°C and oxygen content 5.2-7.5 mg/l** (production test)**:** 1 — survivability, 2 — preserving the physiologically normal state (% of females in this state), 3 —female maturation (% of females in ovulation), 4 — percentage of matured sound females (% of females with fertilized roe of > 50 %), 5 — roe fertilization (one female matured in the control group, 32% of roe fertilization), 6 — sac fry hatching (Ikryaninskiy sturgeon fishery, 1979).

With environment salinity at 5‰, roach producers (50 producers of both genders) and starred sturgeon (10 females) the hematoglobulin and protein levels in blood plasma reduced insignificantly, while salt retention in blood and chamber fluid was maximal. Apparently, this is due to the optimization of the electrolyte balance (Table 1). The electrolyte balance was achieved on account of optimal osmotic gradient between the internal and external environments. This energy-saving gradient ensures preservation of the metabolic and electrolytic homeostasis, i.e. the external environment has biostimulating effect improving body resistance. It is this artificial (modified environment) (NaCl 4-8‰) has the most potential in aquaculture for application in the conditions of close-loop water supply.

1. Physiological state of roach (*Rutilus rutilus caspicus*) and starred sturgeon (*Acipenser stellatus*) in NaCl of varying concentration (Ikryaninskiy sturgeon fishery, 1979)

NaCl, ‰	Reservation timeframe, days	Roach (*n* = 50 producers, ♀ + ♂)		Starred sturgeon (*n* = 10 ♀)		
		levels, *M*±SEM		osmolarity, mosM/l (salinity, ‰)		
		hematoglobulin, g/l (min-max)	total protein, g% (min-max)	blood plasma	cavitary fluid	urine
3	15	6.6±1.1 (5.7-7.95)	9.0±2.95 (7.0-12.9)	–	–	–
5	28	–	6.3±1.5 (4.9-7.9)	164.4 (6.2)	196.0 (7.7)	122.0 (4.5)
	45	9.0±2.95 (7.0-12.9)	6.7±0.7 (5.6-7.0)	–	–	–
12	45	6.3±1.5 (4.9-7.9)	9.0±2.95 (7.0-12.9)	–	–	–
C	34	6.7±0.7 (5.6-7.0)	6.3±1.5 (4.9-7.9)	–	–	–
	28	–	6.7±0.7 (5.6-7.0)	153.0 (5.8)	171.0 (6.6)	155.0 (5.9)

N o t e. C — control group (river water). Gaps mean that the parameter was not defined. The internal environment osmolarity parameters of starred sturgeon were identified and presented by Laboratory of Physiology, Central Research and Development Fishery Institute of the Ministry of Fishery of the USSR.

The fish farm rearing uses the systems of species-specific adaptations only for the river period of life pertaining to the expenditure of financial and power resources and reduction of eurybiontic levels during migration and breeding [2, 13, 14]. The ultimate goal of reproduction (maximum productivity of the population) is achieved in the sea during the feeding period on account of increased survivability and growth. Apparently, fish-rearing needs to use the systems of species-specific phylogenetic adaptations that enable maximum survival rate, productivity and ecological and physiological plasticity of sexual cycles [15]. These systems are most fully implemented during sea feeding period within very narrow ranges of critical salinity [5, 13]. However, in the currently used fishery technology of breeding Atlantic salmon lack stages of creation and maintenance of rearing female stocks, release and allocation of young fish in the feeding water body [6].

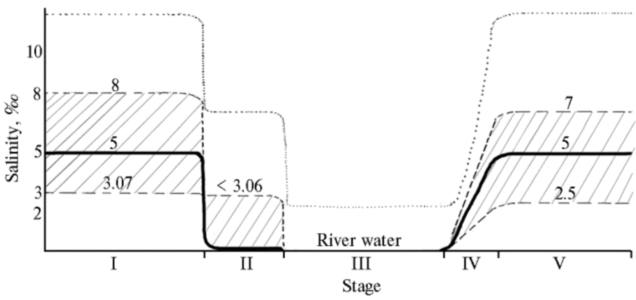

Fig. 3. Salinity patterns at various stages of fishery reproduction of Atlantic salmon (*Salmo salar*) of Baltic population by: I — reservation of female broodstocks in sea cages, II — progeny breeding, III — fishery roe incubation, raising young fish until they are ready to migrate, IV — preadaptation of young fish, V — sea cage breeding of smolts. Unbroken curve means the optimal salinity value, dashed ciurve declares the permitted values, shaded areas are their range; point indicate a curve of anticipated upper values [9].

We have developed a full-cycle salmon reproduction method that includes control of producer reproduction, young fish growth rates and young fish preadaptation to the marine environment [1, 13]. The technology (Fig. 3) includes a wide-scale catching of producers in the sea, maintenance of rearing female stocks in sea cages, breeding and nursing fishery smolts in brackish sea water of critical salinity (AC No. 682197, 965409, patent of the Russian Federation No. 2582347).

2. Comparative fishery and biological parameters of Atlantic salmon (*Salmo salar*) of Baltic population under varying conditions (Leningrad region, 2010-2013)

Parameter	Producers					
	♀+♂		♀		♂	
	sea cages	Nevskiy fishery	sea cages	Nevskiy fishery	sea cages	Nevskiy fishery
Number of individuals	82	163	44	88	32	75
Weight, kg (*M*±SEM)	4.17±0.07	5.00±0.12	3.60±0.05	6.30±0.13	4.40±0.12	2.10±0.14
Mean-square deviation of weight (σ)	0.700	1.616	0.333	1.233	0.700	1.283
Body length (according to Smith), cm (*M*±SEM)	71.60±0.28	74.90±0.71	74.30±0.25	82.00±0.53	63.20±0.04	66.10±0.90
Mean-square deviation of body length (σ)	2.600	9.166	1.683	5.000	0.250	7.833
Fulton's condition factor (Q)	1.02	1.20	1.09	2.60	0.77	1.20
Working fecundity, thousands (*M*±SEM)	–	–	2.40±0.10	4.87±0.03	–	–
Percentage of mature producers (% of maturation)	92.0	84.0	95.0	82.0	97.0	96.0

N o t e. Nevskiy fishery means Nevskiy salmon fishery. The sea cages were located at Alkor-Farm LLC (the Vyborg Bay). Dashes mean that the parameter was not detected.

It is a known fact that during river spawning migration the producers of Atlantic salmon lose their edible qualities and are, therefore, removed from commercial use but continue to be used for artificial reproduction [2, 9]. The presented method allows discontinuing fishing at spawning grounds and growing large viable young fish adapted to the optimal feeding environment, and, ultimately, combine the interests of all reproduction types.

For the first time ripe roe was received and progeny raised to a three-year-old age from producers that maturated naturally in sea cages during the spawning season (October-November) with salinity 2.51-3.06‰ and spawning temperature 3-7°C, with all other conditions being equal with fisheries [2, 13]. The results of working with producers of salmon rearing female stocks using the new technology and comparing them with the parameters received at Nevskiy salmon fishery enabled us to determine identical high fish-rearing and biological parameters (Tables 2, 3). Furthermore, the producers used by fisheries (from spawning offshore areas) displayed several higher fish-rearing parameters and they were more susceptible to the negative impact of thermal anomalies than those of producers in seas cages. J.M. Elliott at al. [16] reported identical findings.

3. Comparative characterization of Atlantic salmon (*Salmo salar*) producers of Baltic population maintained in sea cages (Alkor-Farm LLC, the Vyborg Bay) at Nevskiy salmon fishery in terms of roe maturation quality (Leningrad region, 2010-2013)

Parameter	Sea cages	Nevskiy fishery
Roe fertilization rate, %	92.0	93.4
Roe put for incubation from 1 batch, thousands of units	90-95	475.8
Sperm quality (mobility), points	5	–
Larvae hatching, % of impregnated roe	81.7	89.7

Note. Dashes mean that the parameter was not detected.

The young fish maintained in cages was fed Russian feeds of BimMar company (expenditure 1.3-1.4 kilos). The results of valuation at average water temperature of 3.5 °C, oxygen content of 7-8 mg O_2/l, pH 8-9 (Table 4) indicated that at the ages 1+ and 2+ test young fish maintained in cages increased growth primarily on account of head growth, the length of which increased by 170% while body length increased only by 36%. The body height parameters and fatness coefficient of young fish increased insignificantly, by 35-57%. In the course of analyzing the degree of heterogeneity of individual parameters of young fish we observed that two-year-olds demonstrated the highest variety (1+). Diversity of variation coefficients of two-year-olds reached 23%; for three-year-olds (2+), this parameter was lower and constituted 4.5-17%. This is indicative of declining intensity of development parameters of individuals with age. At the same time, body weight of three-year-olds increased almost by 250%, which points at prevalence of growth processes. This means that upon commencement of smoltification, young fish development is replaced with intensive growth, which corresponds to natural sea feeding [17]. Apparently, the survival rate will increase progressively upon reaching critical salinity 4-8‰ [1, 10, 18]. It is important that this offered method precludes a wide-scale appearance of dwarf males [2, 19]. We believe that the amplified growth and survival rate of young fish if he final fishery cycle is in the sea water are due to the fact that salmon homing is not genetically imprinted [20].

4. Morphometrical parameters of two-year-olds (1+) and three-year-olds (2+) Atlantic salmons (*Salmo salar*) of Baltic population for all batches raised in sea cages (Alkor-Farm LLC, the Vyborg Bay, 2011-2013)

Parameter	*M*±SEM		σ		*Cv*, %	
	1+	2+	1+	2+	1+	2+
Head length (ao), cm	4.60±0.04	7.40±0.04	0.19	0.31	4.20	4.28
Snout length (an), cm	1.80±0.01	2.20±0.02	0.08	0.17	4.77	7.87
Post-orbital head section (po), cm	2.88±0.03	4.20±0.01	0.14	0.09	4.99	2.36
Body length (L), cm	28.70±0.75	39.10±0.22	3.35	1.54	11.66	3.96
Body length (l), cm	26.20±0.43	35.06±0.21	1.93	1.50	7.37	4.29
Maximum body height (gh), cm	6.42±0.06	8.70±0.04	0.31	0.32	4.87	3.76
Minimum body height (ik)	2.19±0.03	3.32±0.03	0.17	0.26	7.89	7.94
Body weight (m), g	280.10±20.08	694.90±14.08	61.34	96.58	17.22	13.91
Fatness coefficient (Q)	1.55	1.61				
Relative growth rate (R)	0.409±0.01	0.490±0.02				

During commercial tests on salmon species, specifically on silver salmon underyearlings *Oncorhynchus kisutsch* (Walbaum, 1792) raised during 1 month in tanks with addition of N-(2-hydroxyethyl)-morpholine or phenethyl alcohol, it was observed that imprinting is formed as late as during the first summer of larvae and young fish raising at fisheries upon transition to active feeding [19, 21]. The release of test young fish and 18-month feeding in the sea resulted in obtaining a bright effect of controllable (obligatory) homing, with 95 and 92%, respectively, of return to the "alien rivers" for each agent. The behavioral response with indications of olfactory imprinting has been observed in sturgeon larvae during transition to active feeding [22]. It is crucial to study behavioral response of sturgeon species for fish-rearing industry and requires a thorough experimental validation based on analysis of species-specific phylogenetic adaptations enabling migration mechanisms [2, 5].

Our findings can be used in a system of measures aimed at preserving rare and endangered fish populations. The idea of their preservation is the underlying idea of a new fish-rearing industry that appeared abroad, i.e. the conservation aquaculture aimed at restoring the natural environment [23]. In Russia, these domains and terms have not been announced or used until now. The conservation aquaculture projects controlled by environment institutions of the USA, Canada and Western Europe include three stages of high-priority action plans: natural reproduction recovery, genetic research and artificial reproduction [23, 24]. These programs are aimed at overcoming a number of negative factors of commercial reproduction of populations, specifically, of domestication of inbred fishery young fish and replacing wild producers with fish-reared with decreased reproductive potential [25-27]. These programs envision ongoing release of fishery-raised young fish when no natural replenishment of the population is available [28]; release of fishery-raised young fish to recover an extinct self-reproducing population [29, 30]; release of fishery-raised young fish to increase the population by supplementation, which is predominant in Russia [8, 31]. This aquaculture domain is based on a combination of effects of natural and fishery reproduction with a focus on recovery of the environmental conditions ensuring natural reproduction of populations [4, 20]. The main objects of these programs in the USA, Canada and Europe are sturgeon and Atlantic salmon populations entered in the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species in the category "endangered species act" [8, 30].

Therefore, industrial sodium chloride solution of critical salinity displays the same biostimulating reservation effects (long-term preservation of fish-rearing quality of producers) as brackish water with the same salt levels. We have successfully reserved the producers of starred sturgeon, Atlantic salmon and roach in critical salinity environment of 4-8‰ with spawning temperature during commercially necessary timeframes. We have determined a possibility to obtain progeny of Atlantic salmon on a wide scale in brackish sea water with salinity up to 3.06 ‰. We have observed the accelerated growth and progressive multiple growth increase of young fish of Baltic salmon population (up to 5-7 times) in brackish sea water of critical salinity in case of perennial sea cage raising. When obtaining progeny from producers in brackish sea water in the areas of feeding and fishing, the load from spawning grounds and commercial dependency of fisheries are released and interests of all types of reproduction and fishing are combined, whereas losses of maintaining broodstocks in optimal reservation environments are minimized. The reduction of processing stages directly at salmon fisheries will allow freeing up additional production capacities in order to improve the efficiency of reproduction. The nursing of young fish in seawater in feeding locations multiplies growth rates, significantly increases young fish survival rate in nature, almost precludes the appearance of "river" dwarf males. Moreover, the smoltification process of young fish shows a wide-scale synchronous nature because it almost identical to natural, and reduces the costs of fishery products. The survival rate of smolts increases due to their preadaptation for release to natural feeding sites.

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