



Manual to determine gonadal maturity of Baltic cod



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PREFACE

This manual has been developed with the purpose to improve the quality of maturity data on Baltic cod, and thereby the basis for estimation of spawning stock size and reproductive potential applied in stock assessment and management. The macroscopic scale used to visually stage gonadal maturity has been evaluated histologically to ascertain and support the determination of stages. Photo documentation of the various stages serves to further increase the accuracy of data by reducing differences in subjective judgement. A plastic laminated version of the manual is focused on its practical application onboard research vessels, where sex and maturity determination is often part of routine sampling to estimate sex ratios and proportion mature in the population.

The macroscopic scale to determine gonadal maturity has been applied by the Danish Institute for Fisheries Research (DIFRES) and the Institute for Marine Sciences (IfM), Kiel, e.g. in the EU-projects: Cod Recruitment in the Baltic (CORE) and Stock Recruitment in the Baltic (STORE). The scale includes 10 maturity stages per sex, which describes the development from juvenile to adult; stages within the annual reproductive cycle; and also abnormalities causing reduced fecundity. For females, ovaries showing different degree of development have been analysed histologically and the macroscopic stages redefined by comparing microscopic characteristics with macroscopic features of the same ovaries as documented by photography. For males, testes have been sampled, but histological analyses have not yet been completed. The revision of the male stages is therefore presently based on development characteristics from photos of the testes as well as shipboard fertilisation experiments to validate the spawning stages. In addition to the female and male scales, a stage describing bisexuality is included.

The gonads analysed and illustrated were sampled during 5 research cruises carried out with R/V Dana in the Baltic Sea. The following people at DIFRES participated in the data collection and contributed in the elaboration of this manual: Jonna Tomkiewicz (concept and project management), Lars Tybjerg (histological analyses), Alex Hansen (photography and layout), Nina Holm (edition and layout), Carl Broberg and Erik Hansen (application and field evaluation). The assistance of Lilian Andersen (DFU) and the crew onboard R/V Dana is appreciated. The support of Dr. Åse Jespersen, Zoological Institute, Copenhagen University, Denmark, in histological analyses and the useful remarks on the manuscript made by Joanne Morgan, Dept. of Fisheries and Oceans, Aquatic Resources Division, St. Johns, Canada, and David Stokes, Marine Institute, Dublin, Ireland, are acknowledged. The manual has been financially supported by the Danish Ministry of Food, Agriculture and Fisheries via the project: Growth variation of Cod Stocks in Danish Waters (FISK-09) and by the European Commission via the projects: CORE (AIR2-CT94-1226) and STORE (FAIR-CT98-3959).

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Charlottenlund, December 2002

INTRODUCTION

A. Reproductive cycle and maturity stages

The reproduction of Baltic cod (ICES Sub-division 25-32) is characterised by the occurrence of sexual maturation at a young age in both sexes, as well as an extended spawning season ranging from March to September (Brander, 1994; Tomkiewicz *et al.*, 1997; Tomkiewicz & Köster, 1998). Fecundity is also high (Kraus *et al.*, 2000) with large hydrated eggs (Thorsen *et al.*, 1996) relative to other cod stocks. These features are generally perceived as adaptations to the variable hydrographic environment in the Baltic Sea which, being a brackish water area, is borderline for cod distribution.

The reproductive cycle and gonadal development segregate into different stages and phases with specific characteristics. The applied maturity scale includes 10 stages per sex as well as a bisexual stage. The scale is based on an 8 level scale defined by Maier (1908). Maier's scale is a general one for fish, while the present scale is specific for cod. Two stages have been added to identify specimens in resting condition (Stage IX), specifically spawning omission, and reproductive malfunction (Stage X). The reproductive stages are described in Table 1 and the illustrated pages thereafter. The 10 stages can be grouped into 5 phases: I. Juvenile; II. Preparation; III-IV. Maturation; V-VII. Spawning; VIII-IX. Regeneration; and X. Degeneration. The figure below illustrates the stages and phases in relation to the reproductive cycle:

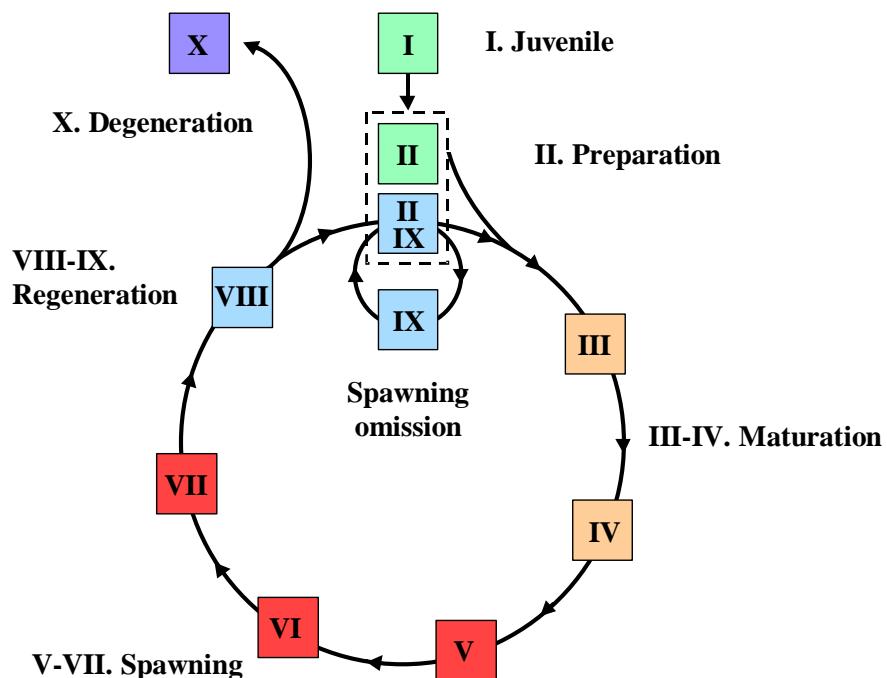


Figure 1. Developmental stages and phases defined histologically and macroscopically from ovaries of cod sampled in the Baltic Sea.

Gonads used to validate the maturity scale were sampled on 5 research cruises in the Baltic Sea (ICES Sub-div. 25, 26 and 28) during 1998 and 1999. The cruises were conducted at different times of the year (November-December, February-March and July), which provided the opportunity to sample gonads at different developmental stages. The sampling was targeted such that a range of different sized cod was covered for each maturity stage. Length, round and gutted weight of sampled fish was recorded and the gonad was weighed, measured and photographed prior to preservation. Ovaries have subsequently been analysed microscopically and the results used to define morphological criteria for the different stages and phases (Tomkiewicz *et al.*, 2003). The description of macroscopic criteria has been revised by comparing the histological results with the photographic records of the gonads. The microscopic analysis of testes has not yet been completed and the redefinition of stages is based on macroscopic characters from photos and fertilisation experiments on board R/V Alkor. The latter showed that the fertilisation success of males in Stage V was equal to those in Stage VI thereby confirming that Stage V as well as VI and VII is a spawning stage (Tomkiewicz & Köster, unpublished data). Bisexuality, which is a rare abnormality in cod, has been described as a separate stage. One of the illustrated gonads originates from one of the research cruises, while the other was provided by a fisherman from the Danish island, Falster, and originates from the western Baltic Sea (ICES Sub-division 24).

B. Application of the manual

Stage description

The relative size and location of gonads introduces the stage definition. The features relating to gonad consistency and structure that follow are the primary characters in determination of maturity stage. Changes in colour that occur during development due to e.g. yolk and sperm formation, presence of hydrated eggs and semen, are also described. However, colour is a secondary trait as variation within stages is considerable and reproduction of colours from photos is subjected to error. The appearance of eggs, features of the lumen and other visible characteristics supplement the description. Normal size limits of fishes and range of the gonadosomatic index ($GSI = \text{gonad weight/cleaned weight} \times 100$) specifying gonad weight relative to body weight are also included where relevant.

Illustrations and text boxes

Each maturity stage is illustrated by 4 examples. These gonads are characteristic for the stage, but morphological variation within stages is also considered. A small text box indicates total length (L_t) and round weight (W_t) of the fish, length (L) and weight (W) of the gonad, sampling month (M), and fish identification number (Id). Specimens of different sizes are illustrated to represent the size range of each stage. The degree of development varies somewhat within each stage with a gradual change in appearance of gonads over time in relation to the annual cycle. The sampling time of gonads illustrated broadly represent the seasonal changes in development for Baltic cod.

Lower limits for sex and maturity determination

Sex can be distinguished macroscopically in cod from app. 10 cm, but is time consuming for smaller specimens because testes are very small and difficult to identify. For routine sampling, a lower limit of 15 cm is therefore recommended and specimens below this limit will, independent of sex, be juvenile (Stage I). Sex determination of all

sampled specimens above this limit should be aimed achievable, because females are more easily identified than males. If difficult specimens are regularly omitted, a skewed sex ratio with female dominance of the smaller size groups may result.

C. Utility in relation to assessment

The optimal sampling time for maturity data to estimate spawning stock size and biomass is shortly before the start of the spawning season, i.e. February-March for Baltic cod. At this time, spawning migration has not started, which simplifies stock coverage and sampling design, but ripening of most specimens that will spawn the following season has progressed to vitellogenesis or spermatogenesis (Tomkiewicz & Köster, 1999). The commencement of ripening improves the ability to separate spawners from immature and resting specimens (spawning omission). In autumn and early winter, the proportion of the stock that will participate in the next spawning season cannot be accurately determined (Tomkiewicz *et al.*, 2003). At this time, not all potential spawners have started ripening and the development of specimens in early ripening stage (III) is often not so progressed that they can be accurately identified.

A spawning probability function defines the proportion per age or length group that can be assumed to spawn in the following spawning season. The proportion of spawners includes specimens in stages III, IV, V and VI, while the proportion of non-reproducers includes specimens in stages I, II, VII, VIII, IX, X and XX. Specimens in the former category sampled in February-March would, had they survived until spawning, have contributed to reproduction, whereas this is unlikely for specimens in the latter category. Specimens in stages, X and XX, may spawn, but their fecundity is reduced and physiological or behavioural changes may influence their ability to successfully reproduce. Spawning probability is a more precise expression to estimate spawning stock size than a maturity ogive that indicates the proportion of adult specimens in the stock, i.e. the proportion in stages III-X relative to I-II (Tomkiewicz *et al.*, 2003).

D. References

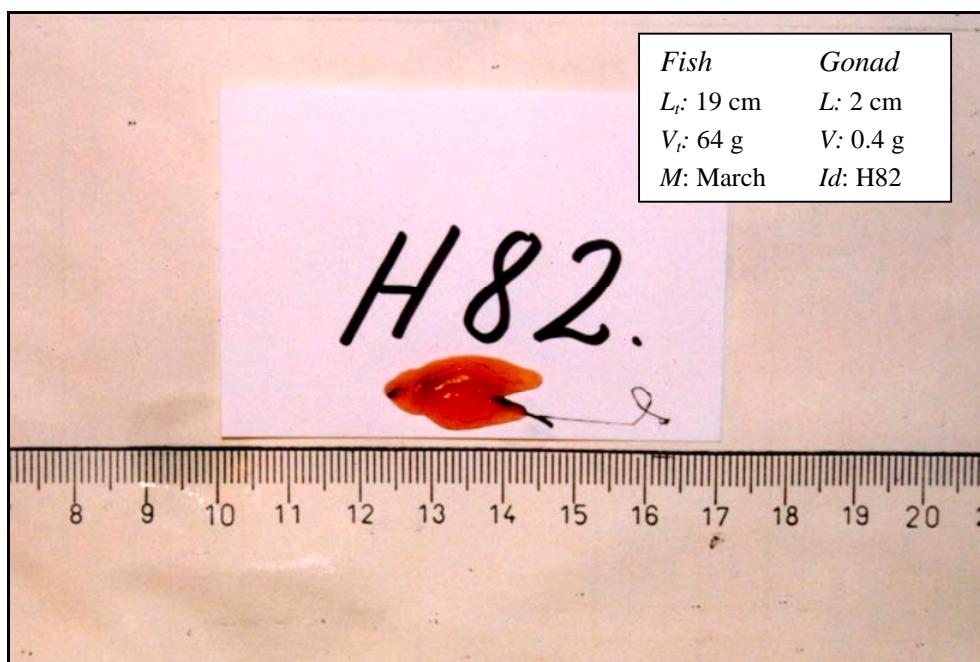
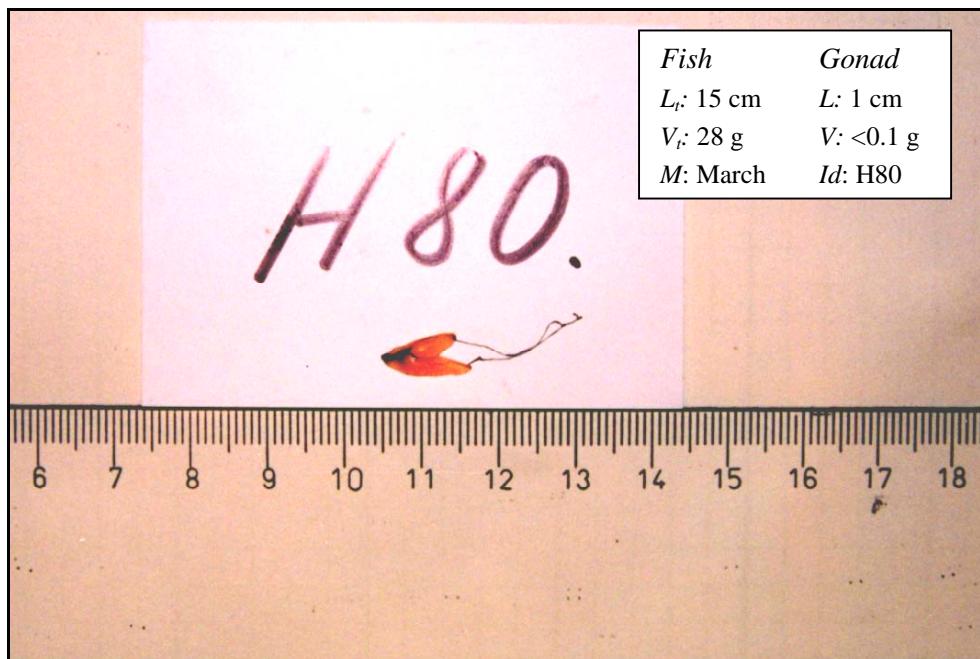
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Stage	Macroscopic characters to determine gonadal maturity of females
I	Juvenile Ovaries emerge as tiny, paired organs close to bladder; glassy transparent to orange-reddish translucent in larger specimens. L_T rarely above 30 cm; $GSI < 1$.
II	Preparation Ovaries small, but easily distinguishable posterior in body cavity; soft with even surface (flattens on a solid sheet); blurred translucent, reddish-orange. L_T : 25-60cm; $GSI < 1.5$.
III	Ripening 1: Oocyte recruitment Ovaries still small and restricted to posterior body cavity; firmer than II and roe shaped (keep form on a solid sheet), surface uneven; opaque orange-red to dark orange with greyish cast in large females. Tiny opaque oocytes emerge towards end of stage. L_T rarely below 30 cm; GSI : 1-7.5.
IV	Ripening 2: Late vitellogenesis Ovaries enlarged to mid body cavity; plump and firm with prominent blood vessels; opaque, orange to creamy yellow. Oocytes clearly visible and densely packed. GSI : 3-14.
V	Spawning 1: Initiation of spawning Ovaries extending into anterior body cavity; distended and soft; opaque, orange to creamy yellow. Single glassy, hydrating oocytes among abundant opaque, vitellogenic oocytes (as in IV, but round and larger). Viscous fluid or hydrated eggs in lumen may occur. GSI : 12-25.
VI	Spawning 2: Main spawning period Ovaries fill most of body cavity; very distended and soft; appear granulated orange- to reddish-grey from mixture of opaque and glassy oocytes. Lumen containing viscous fluid in excess or hydrated eggs. GSI : 15-60.
VII	Spawning 3: Cessation of spawning Ovaries shrunk to posterior body cavity; flabby with prominent blood vessels; unclear reddish- grey. Hydrated oocytes present; opaque oocytes few or absent. Lumen with excess fluid and frequently hydrated eggs. GSI : 3-8.
VIII	Regeneration 1: Spent Ovaries contracted; slack with greyish cast; rich in blood vessels; dim translucent reddish-grey. Vitellogenic oocytes absent, but single hydrated eggs or atretic oocytes (opaque, irregular granules) may occur. GSI normally 2-3; with atresia up to 10.
IX	Regeneration 2: Resting and spawning omission Ovaries small as in II, but with signs of previous spawning; e.g. greyish cast and somewhat uneven walls; blurred translucent, reddish-grey, but more granulated and opaque than in II. GSI : 1-3.
X	Degeneration: Reduced fertility A: Ovaries with fibrous tissue formation; affected areas compact and hard, brownish-yellow opaque; non-affected parts with normal development. Observed in females from 65 cm. B: Other abnormalities.

Stage	Macroscopic characters to determine gonadal maturity of males
I	Juvenile Testes emerge as a pair of thin strings along air bladder. Lobules tiny, glassy transparent to reddish translucent in larger specimens. L_T rarely above 30 cm; $GSI < 0.1$.
II	Preparation Testes small, but distinguishable along air bladder. Lobules small, blurred translucent and reddish. L_T : 20-50cm; GSI : 0.1-0.5.
III	Ripening 1: Early spermatogenesis Testes still small, close to air bladder. Lobules plump and soft, rich in blood vessels, completely or partially opaque, reddish. L_T rarely below 20 cm; GSI : 0.5-6.
IV	Ripening 2: Late spermatogenesis Testes enlarged and prominent dorsal in body cavity; Lobules plump and brittle; reddish-white. Empty, transparent spermatducts with prominent blood vessels; no sperm release. GSI : 1-18.
V	Spawning 1: Initiation of spawning Testes extending into ventral part of body cavity. Lobules distended and brittle, opaque creamy-white. Spermatducts filled with viscous semen and a viscous droplet may be released from vent. GSI : 3-22.
VI	Spawning 2: Main spawning period Testes large and prominent in body cavity (as in V). Lobules still plump, but soft; completely opaque, whitish. Spermatducts filled with fluid, milky semen that easily flows from vent. GSI : 3 to 25.
VII	Spawning 3: Cessation of spawning Testes shrunk to dorsal part of body cavity; soft and flabby. Lobules almost empty, opaque, reddish-white. Spermatducts still with fluid semen that easily flows from vent. GSI : 0.5 to 4.
VIII	Regeneration 1: Spent Testes contracted, close to air bladder; rich in blood vessels. Lobules empty, flabby, reddish potentially with a greyish cast. Spermatducts with signs of previous distension, often with visible remains of semen. $GSI > 1.5$.
IX	Regeneration 2: Resting and spawning omission Testes small (as in Stage II), but with signs of previous spawning; e.g. lobules slightly larger than in II; spermatducts often with greyish cast. $GSI < 1.5$.
X	Degeneration: Reduced fertility A: Testes with adipose tissue formation; affected parts undeveloped, hard, yellowish; non-affected parts with normal development. Observed in males from 50 cm. B: Other abnormalities.

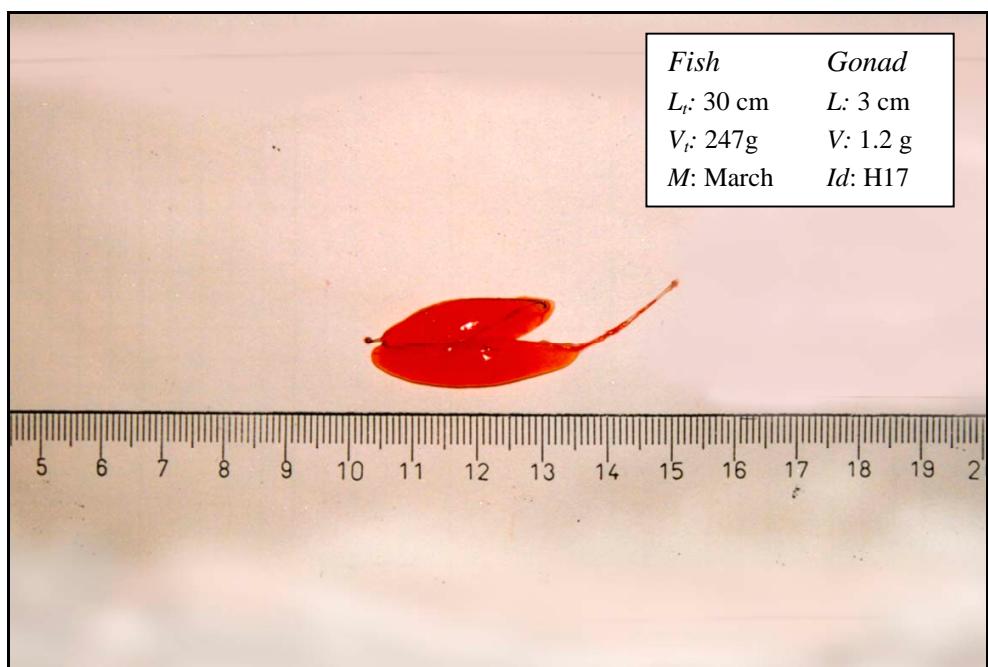
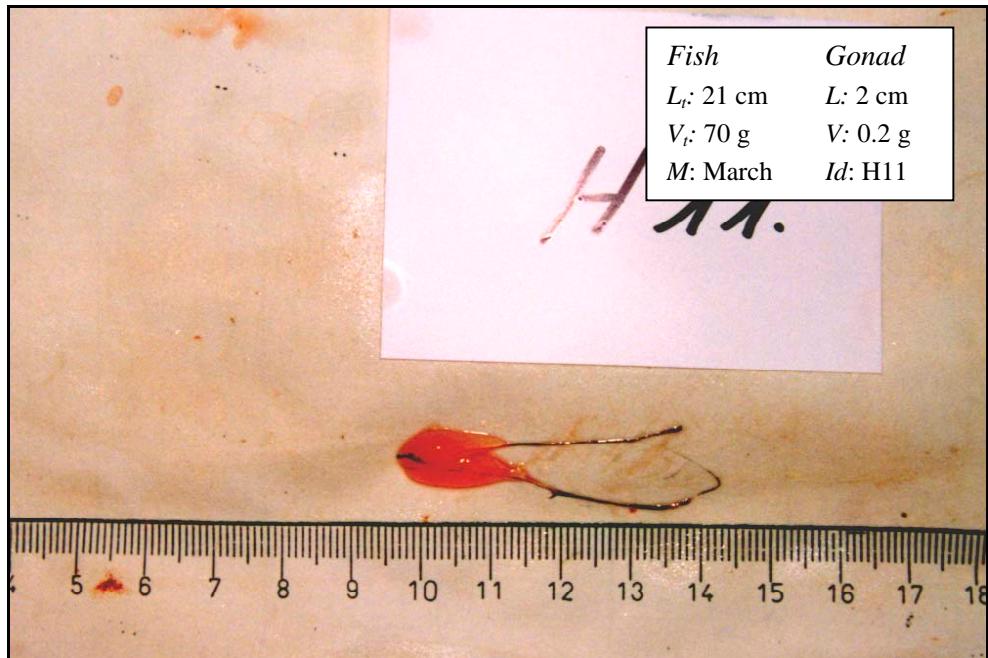
I. Juvenile

Ovaries emerge as tiny, paired organs posterior in body cavity close to bladder; glassy transparent to orange-reddish translucent in larger specimens. L_T rarely above 30 cm; $GSI < 1$.



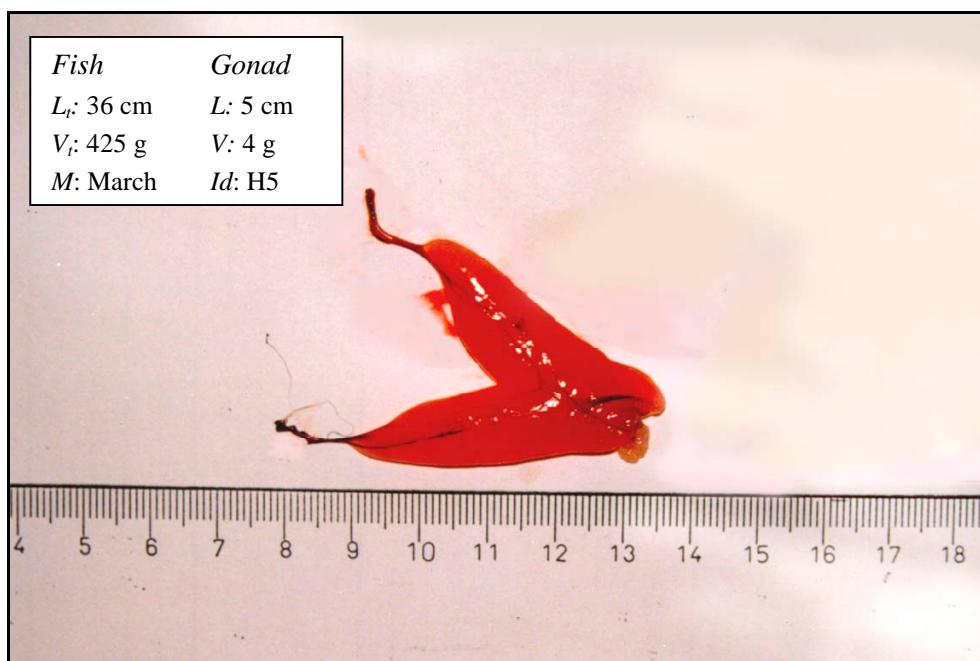
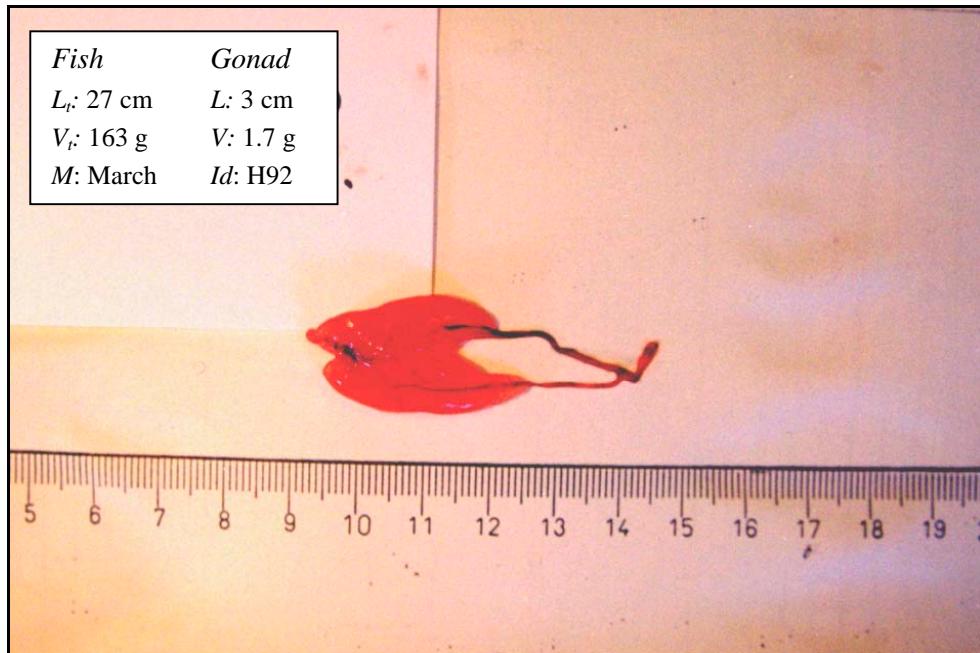
I. Juvenile

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II. Preparation

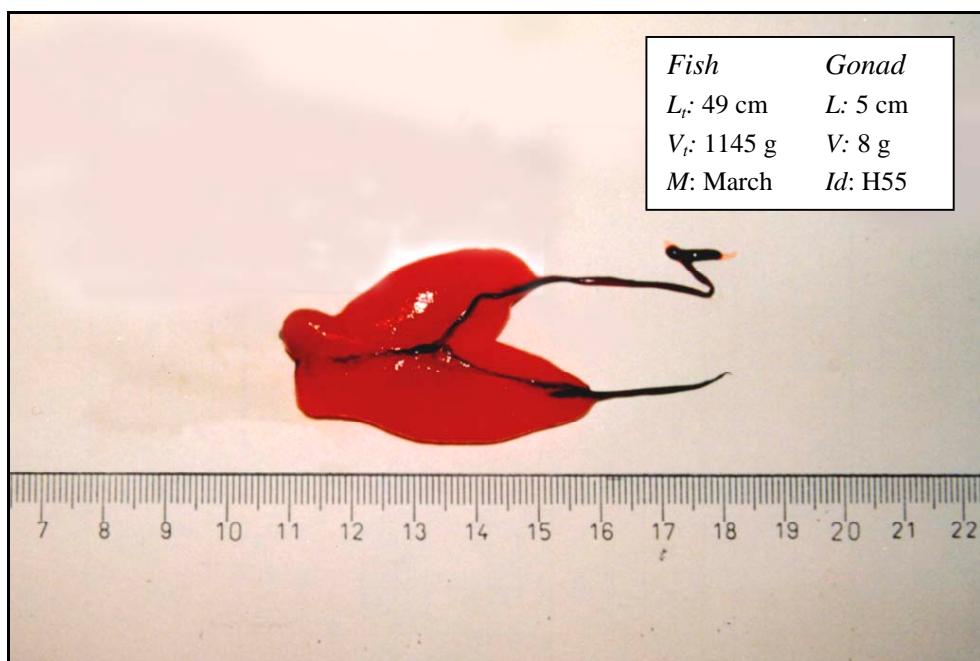
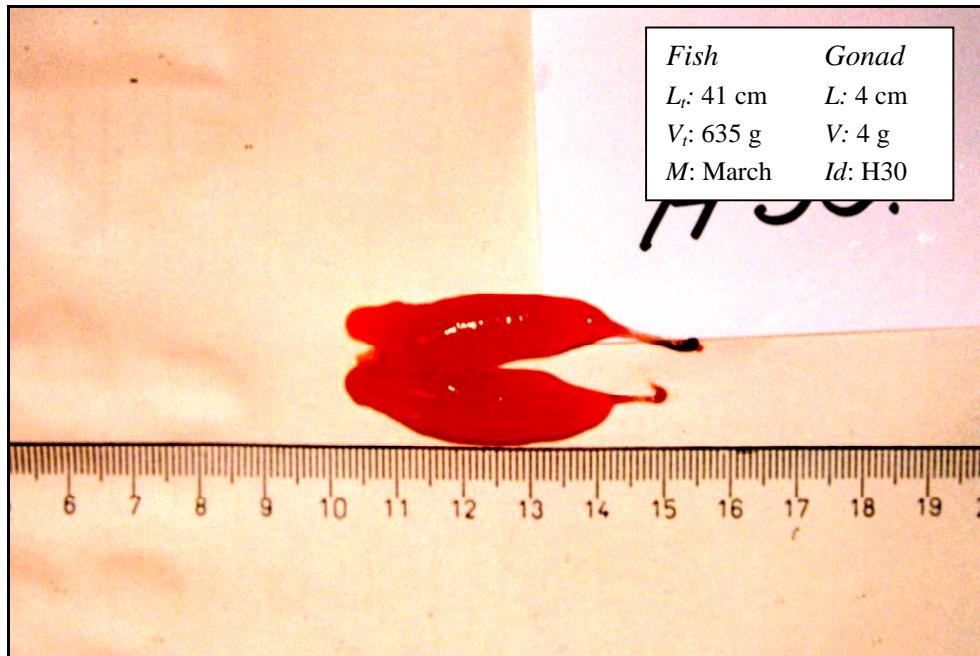
Ovaries small, but easily distinguishable posterior in body cavity; soft with even surface (flattens on a solid sheet); blurred translucent, reddish-orange.
 L_T : 25-60cm; $GSI < 1.5$.



II. Preparation

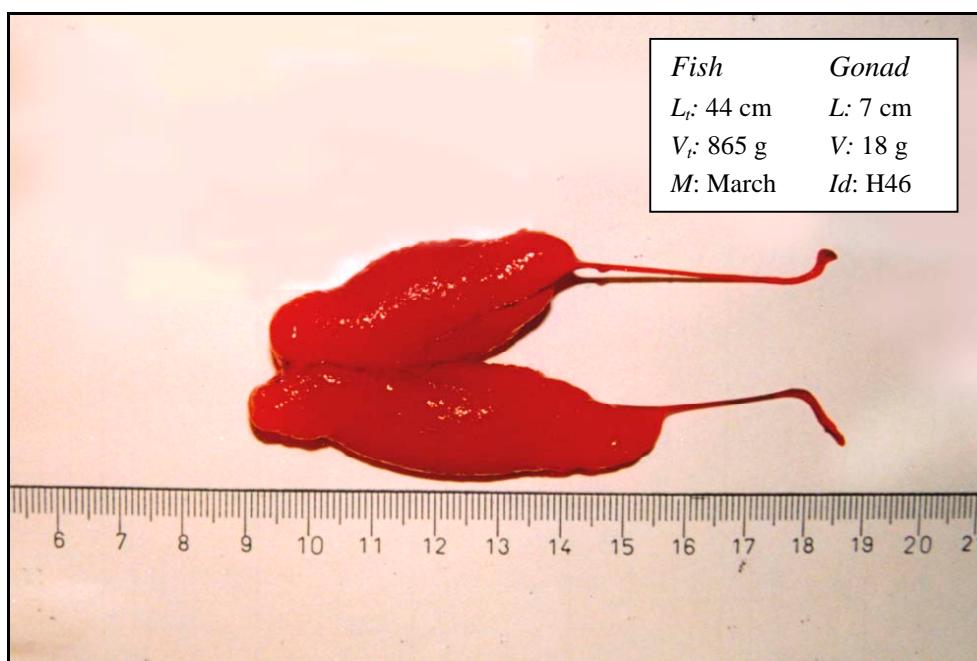
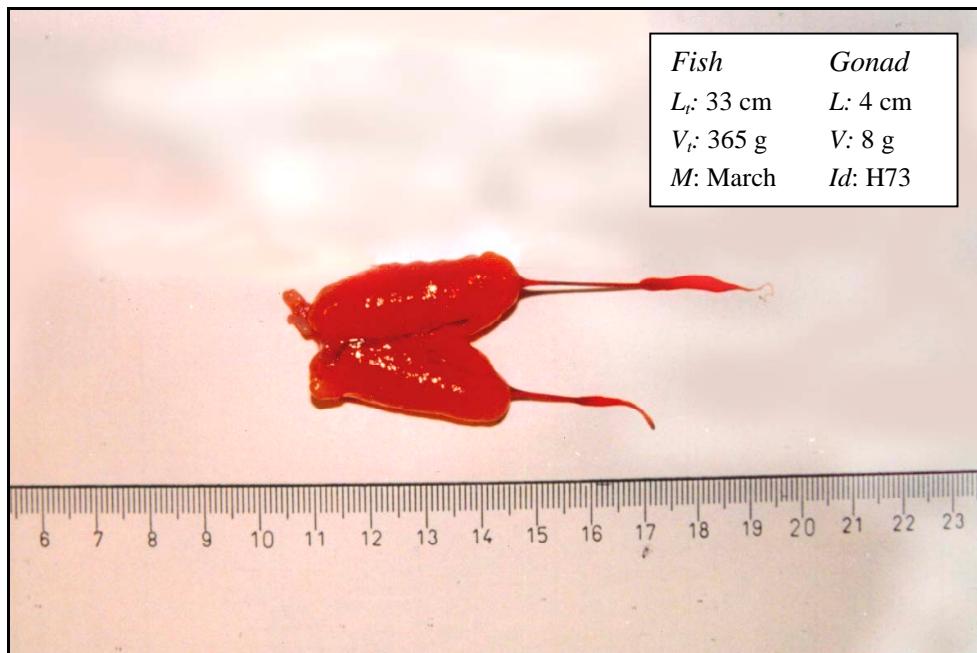
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II



III. Ripening 1: Oocyte recruitment

Ovaries still small and restricted to posterior body cavity; firmer than II and roe shaped (keep form on a solid sheet), surface uneven; opaque orange-red to dark orange with greyish cast in large females. Tiny opaque oocytes emerge towards end of stage. L_T rarely below 30 cm; GSI: 1-7.5.

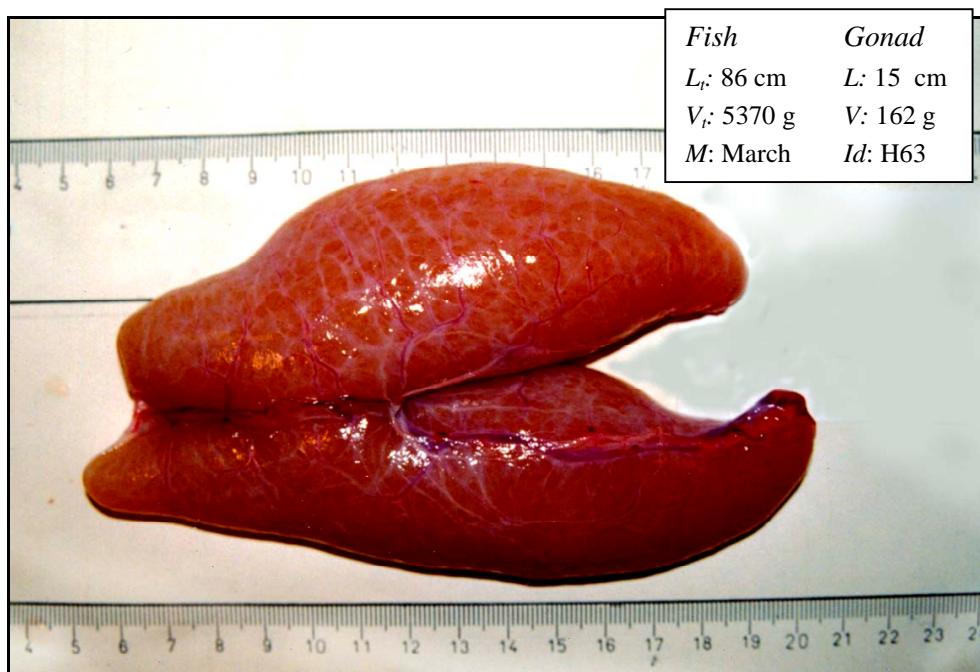
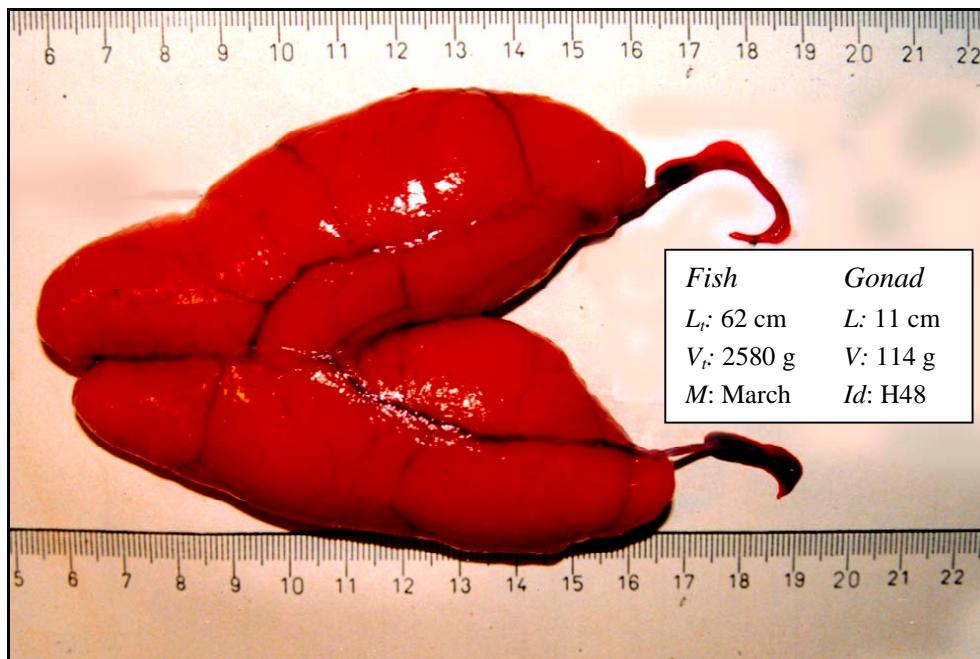


III. Ripening 1: Oocyte recruitment

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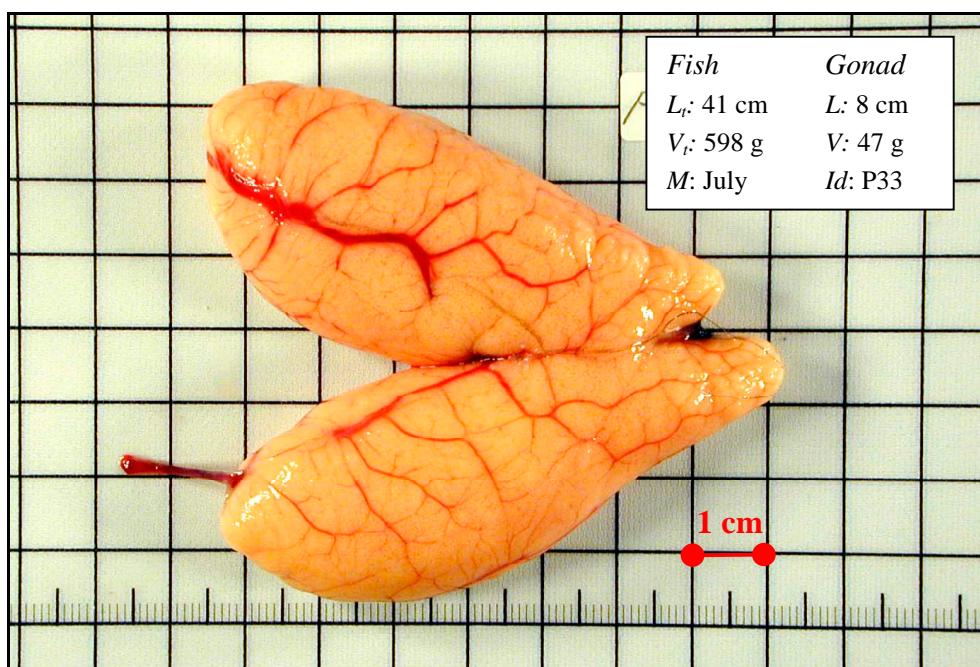
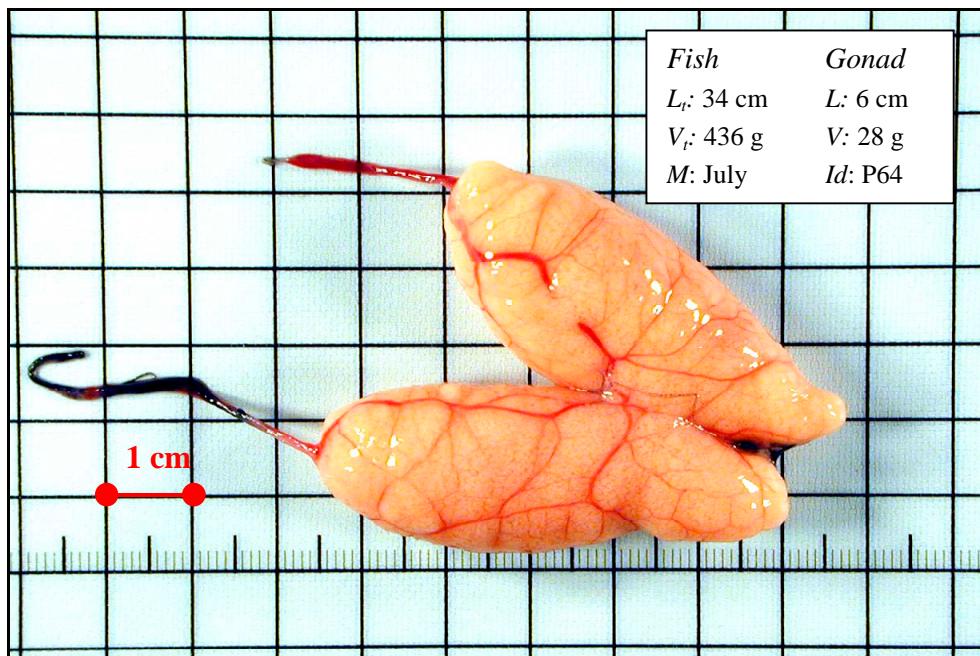
Tip! Ovaries of larger females often have a greyish cast reducing the ability to separate Stage III from IX (Resting). The orange shine of the tissue indicating yolk formation is easier to see, if the ovary is cut open.

III



IV. Ripening 2: Late vitellogenesis

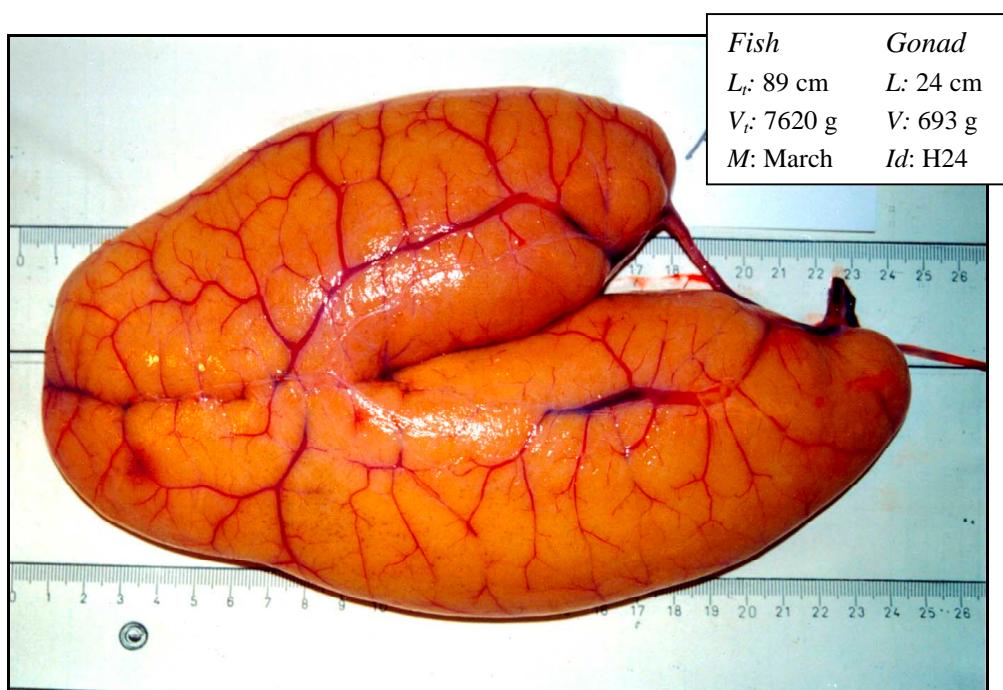
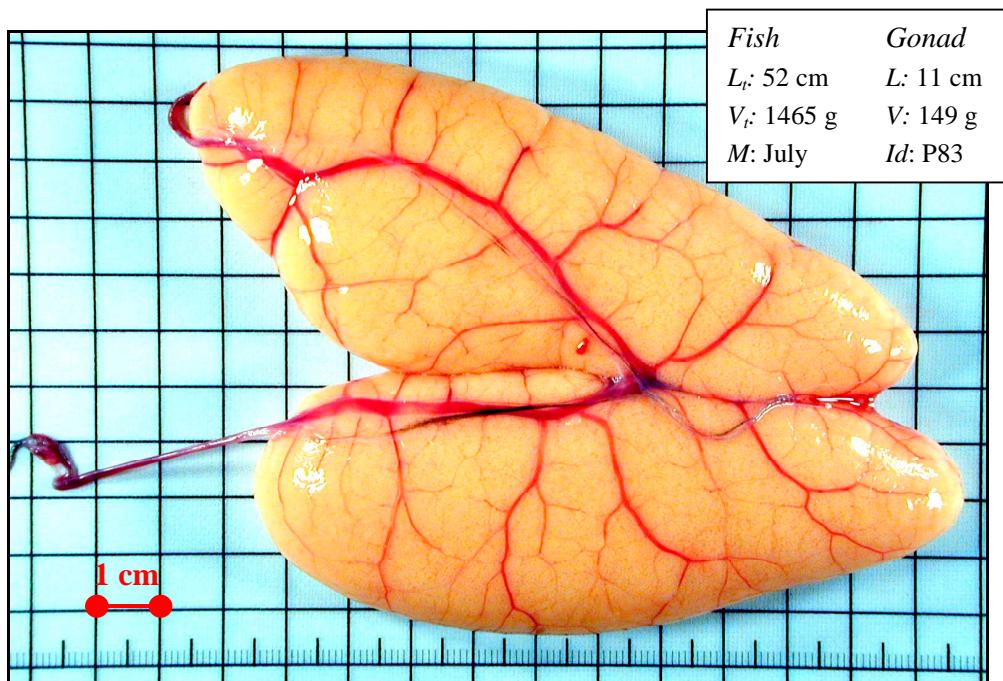
Ovaries enlarged to mid body cavity; plump and firm with prominent blood vessels; opaque, orange to creamy yellow. Oocytes clearly visible and densely packed. GSI: 3-14.



IV. Ripening 2: Late vitellogenesis

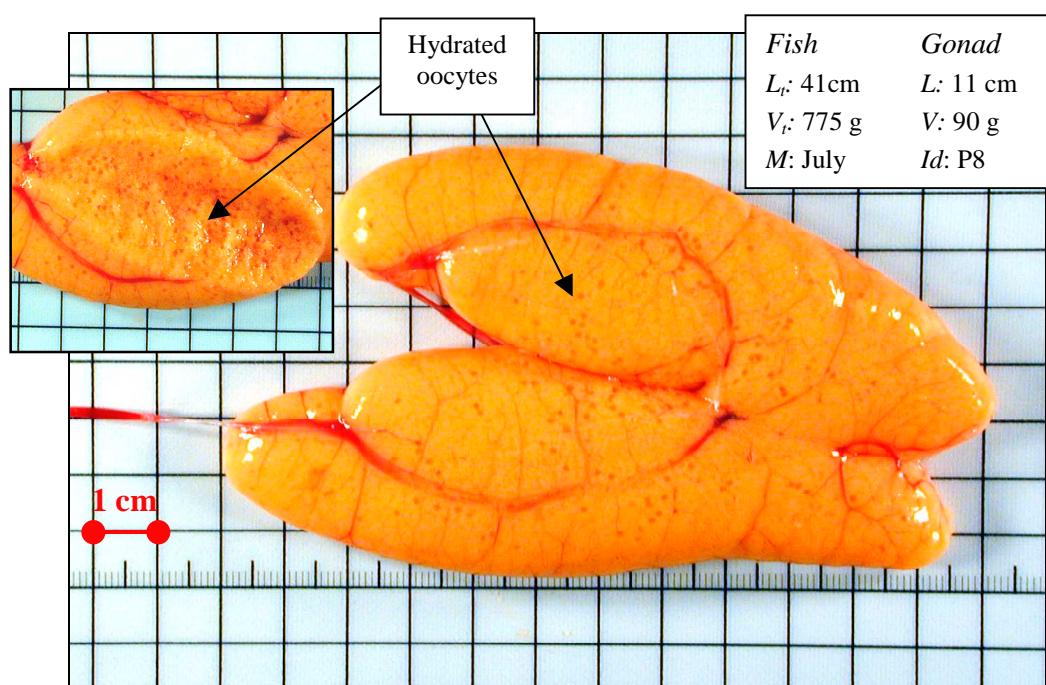
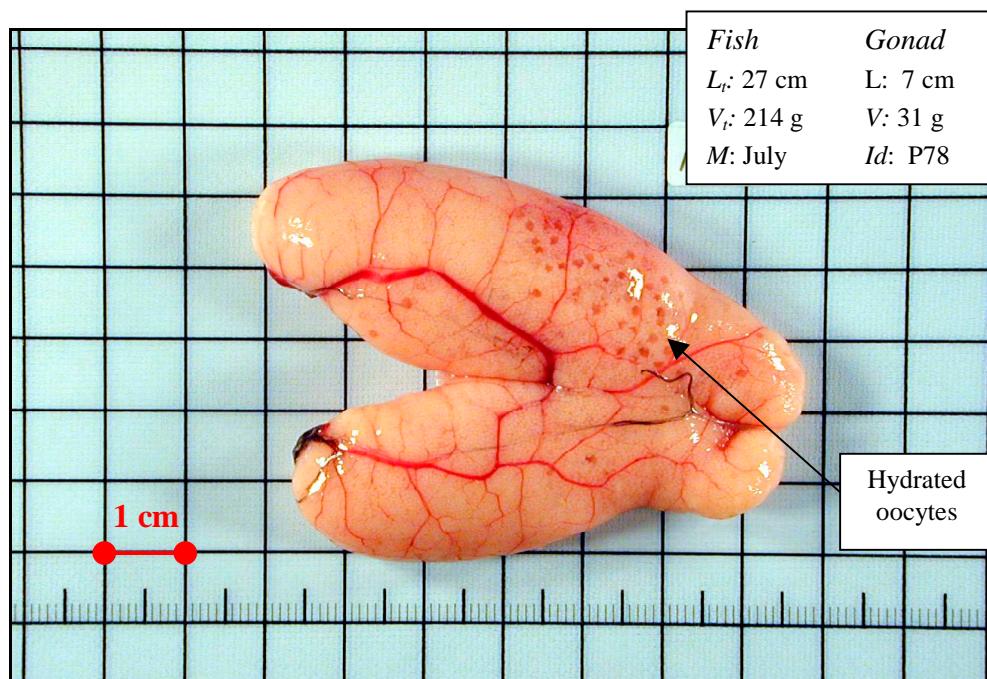
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IV



V. Spawning 1: Initiation of spawning

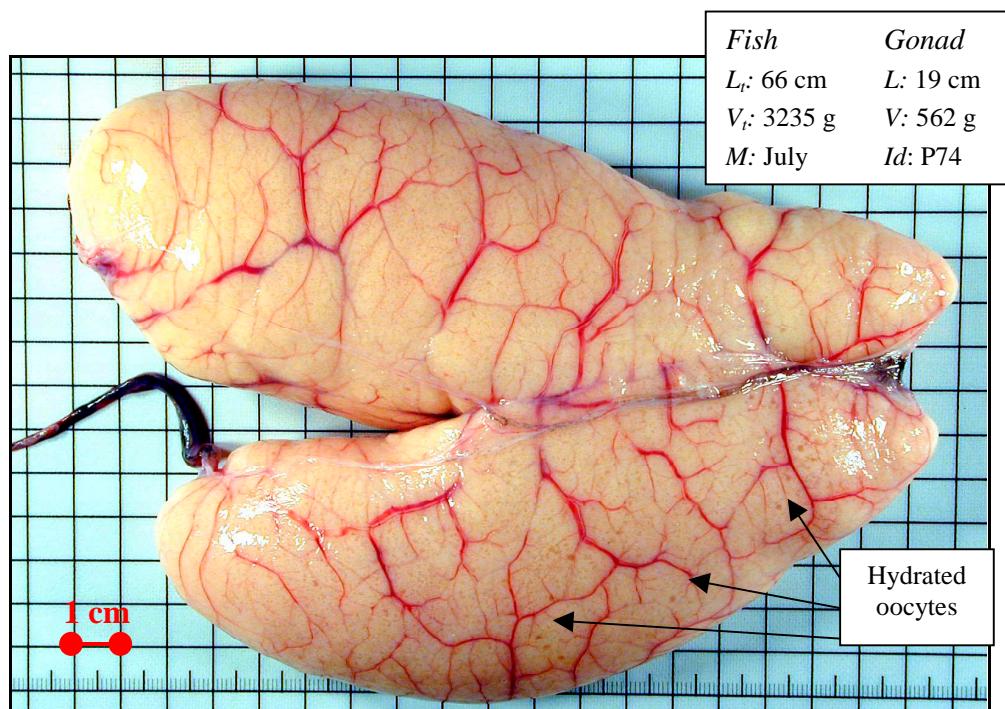
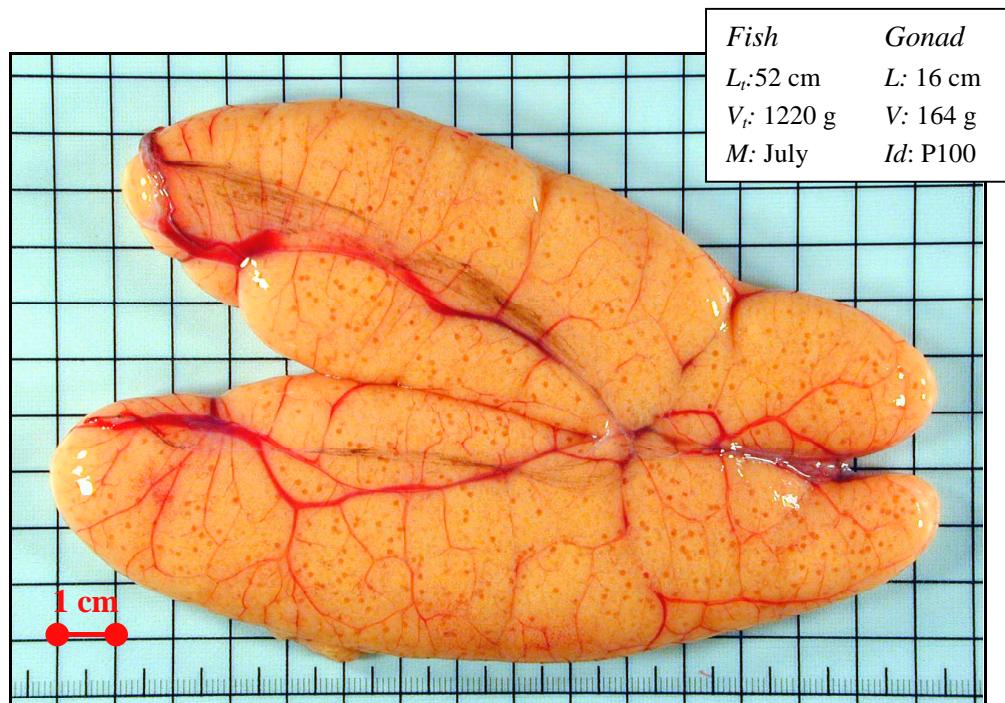
Ovaries extending into anterior body cavity; distended and soft; opaque, orange to creamy yellow. Single glassy, hydrating oocytes among abundant opaque, vitellogenic oocytes (as in IV, but round and larger). Viscous fluid or hydrated eggs in lumen may occur. GSI: 12-25.



V. Spawning 1: Initiation of spawning

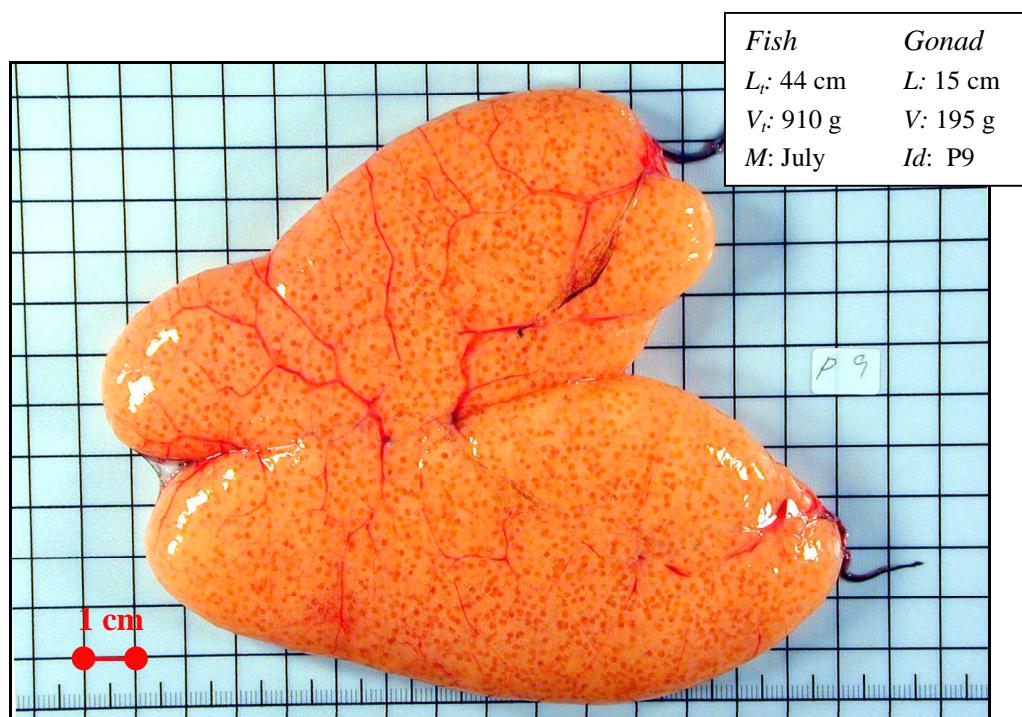
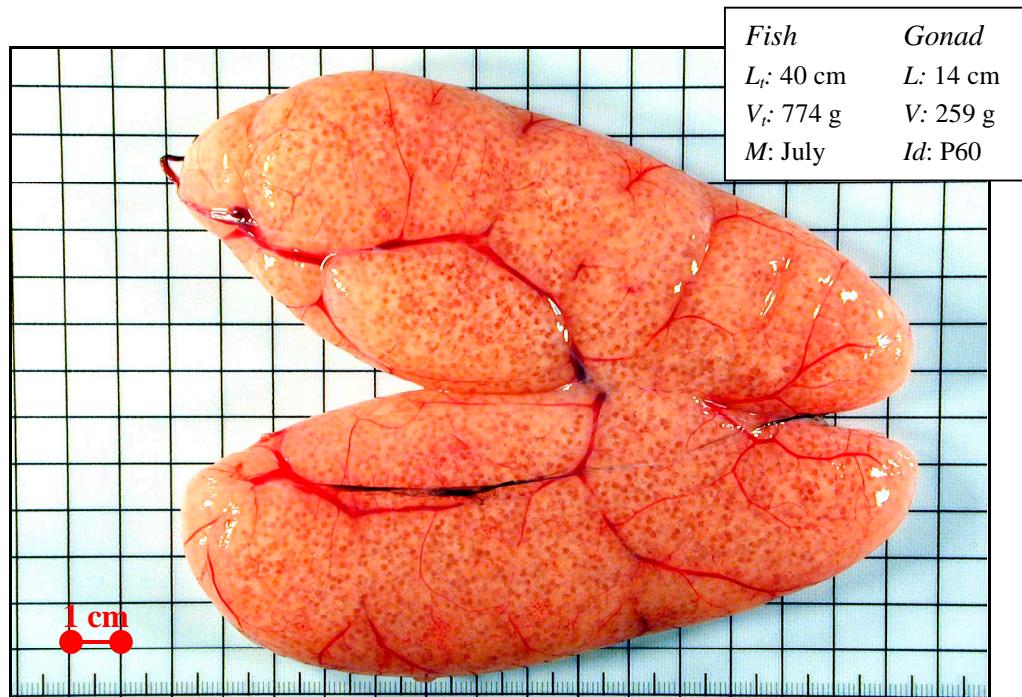
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Tip! In case of doubt, Stage V can be separated from IV by cutting the ovary open – if lumen contains viscous fluid, the ovary is Stage V.



VI. Spawning 2: Main spawning period

Ovaries fill most of body cavity; very distended and soft; appear granulated orange- to reddish-grey from mixture of opaque and glassy oocytes. Lumen containing viscous fluid in excess or hydrated eggs. GSI: 15-60.



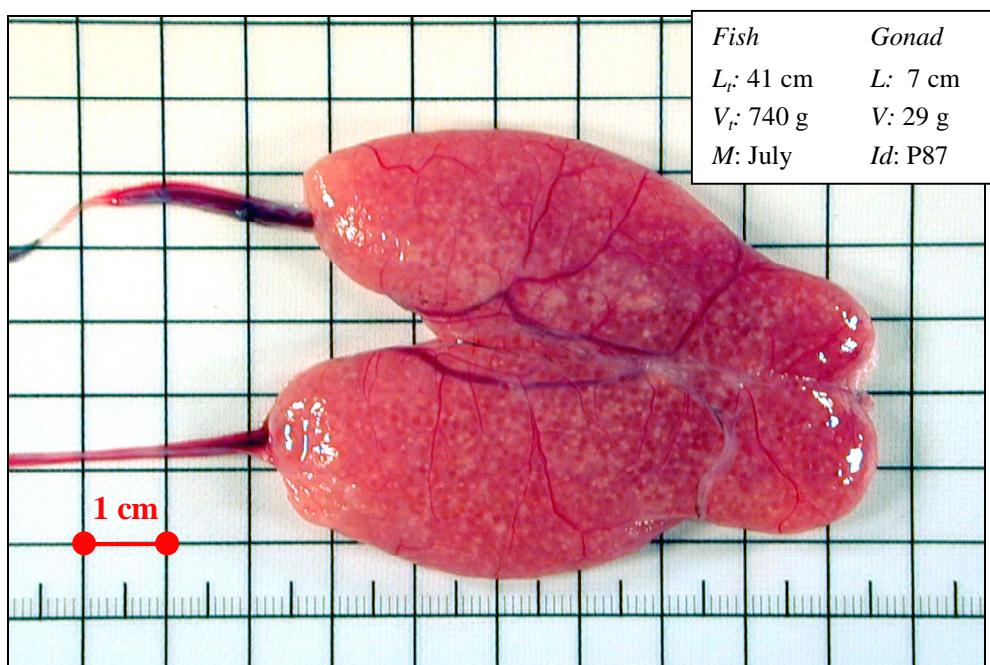
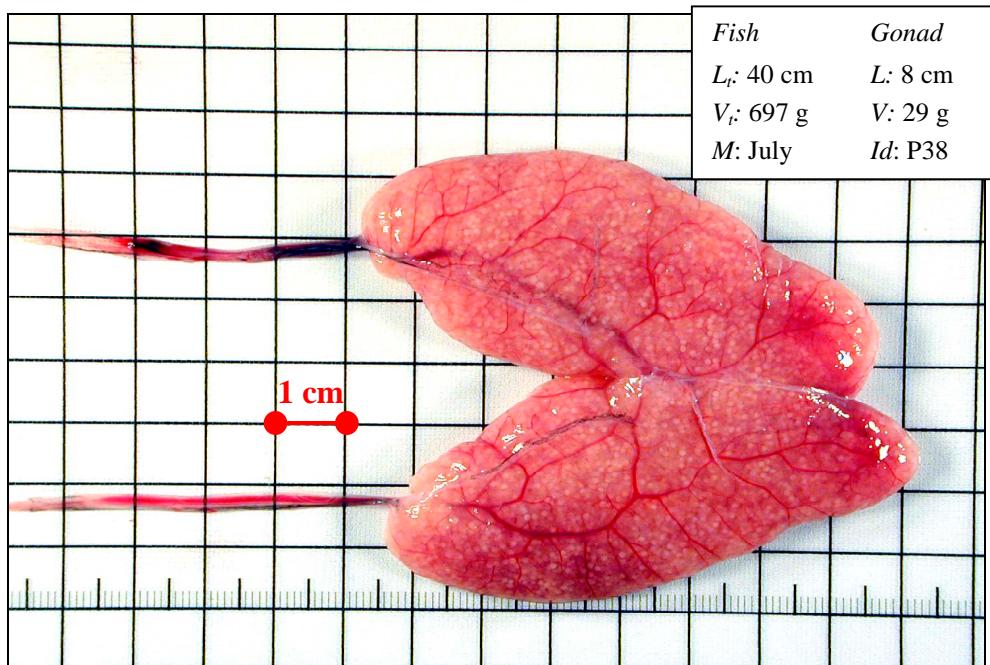
VI. Spawning 2: Main spawning period

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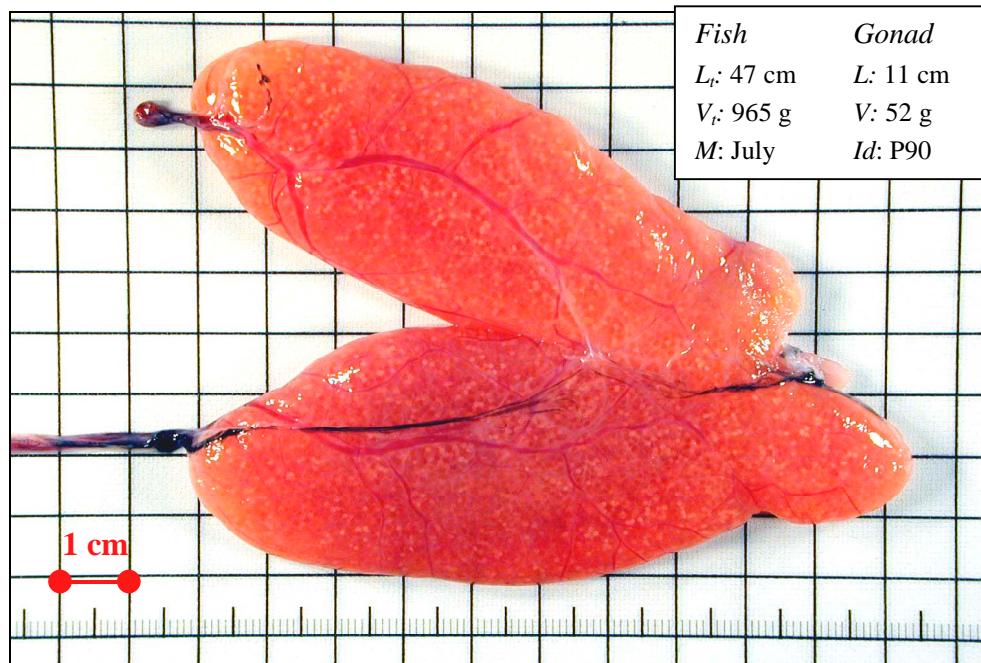
VII. Spawning 3: Cessation of spawning

Ovaries shrunk to posterior body cavity; flabby with prominent blood vessels; unclear reddish- grey. Hydrated oocytes present; opaque oocytes few or absent. Lumen with excess fluid and frequently hydrated eggs. GSI: 3-8.



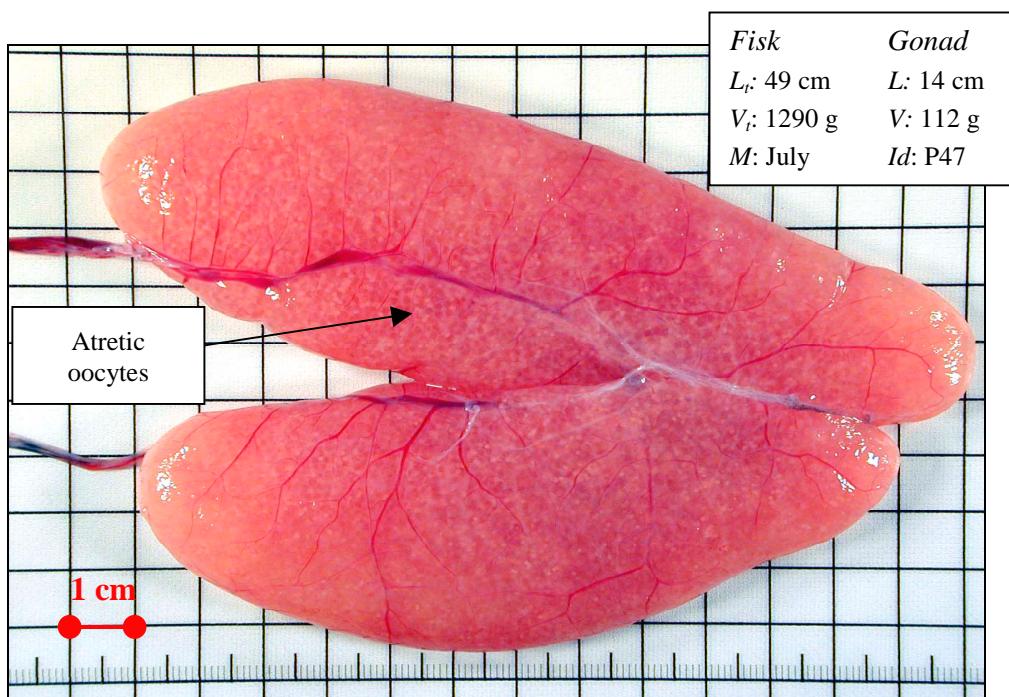
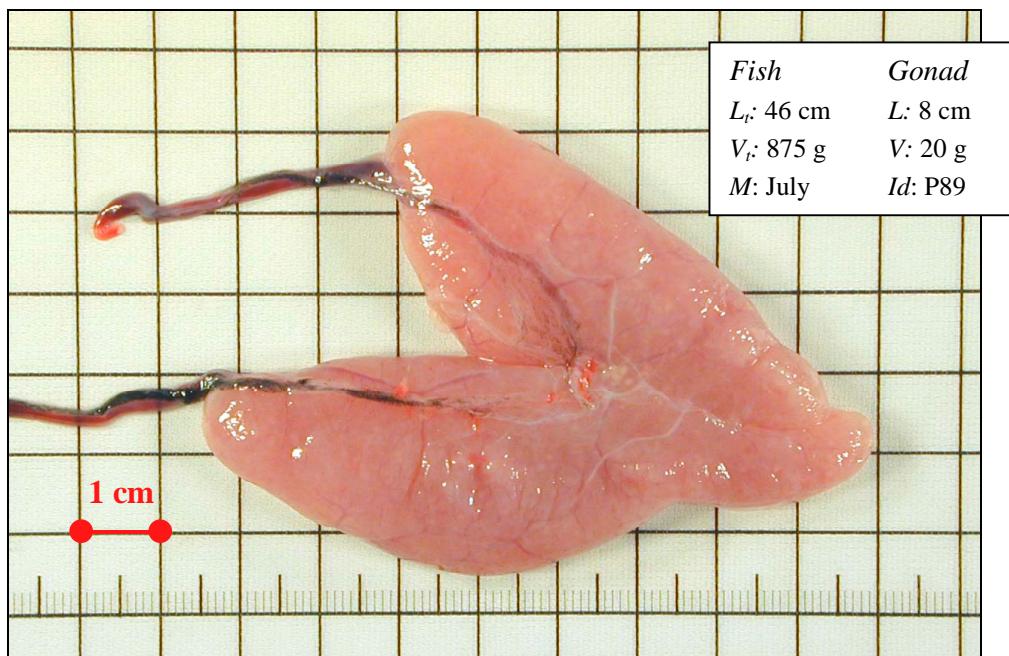
VII. Spawning 3: Cessation of spawning

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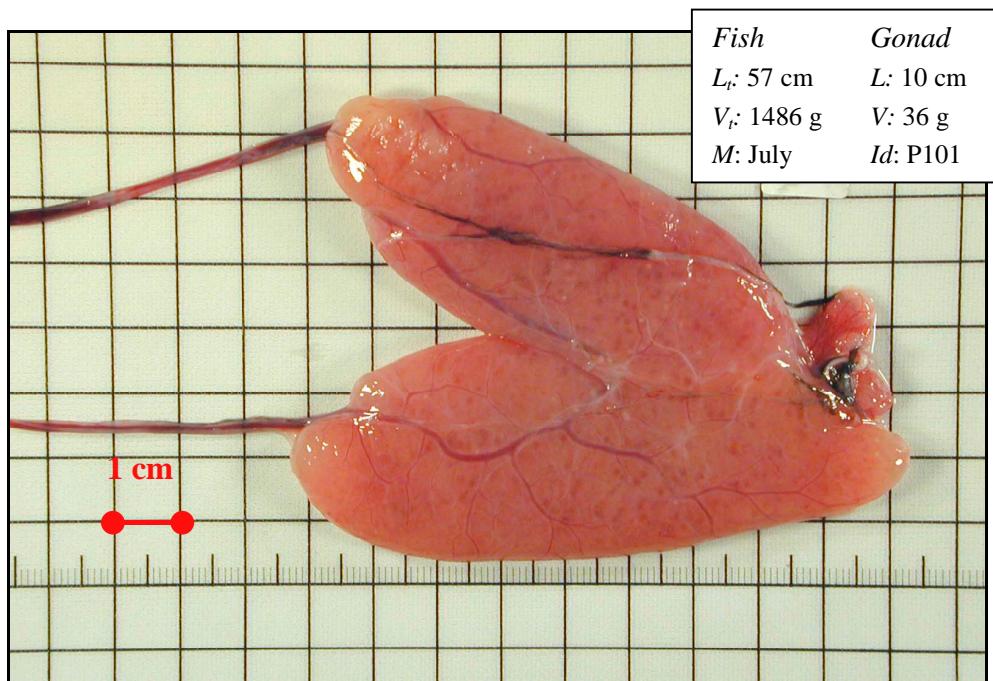
VIII. Regeneration 1: Spent

Ovaries contracted; slack with greyish cast; rich in blood vessels; dim translucent reddish-grey. Vitellogenic oocytes absent, but single hydrated eggs or atretic oocytes (opaque, irregular granules) may occur. GSI normally 2-3; with atresia up to 10.

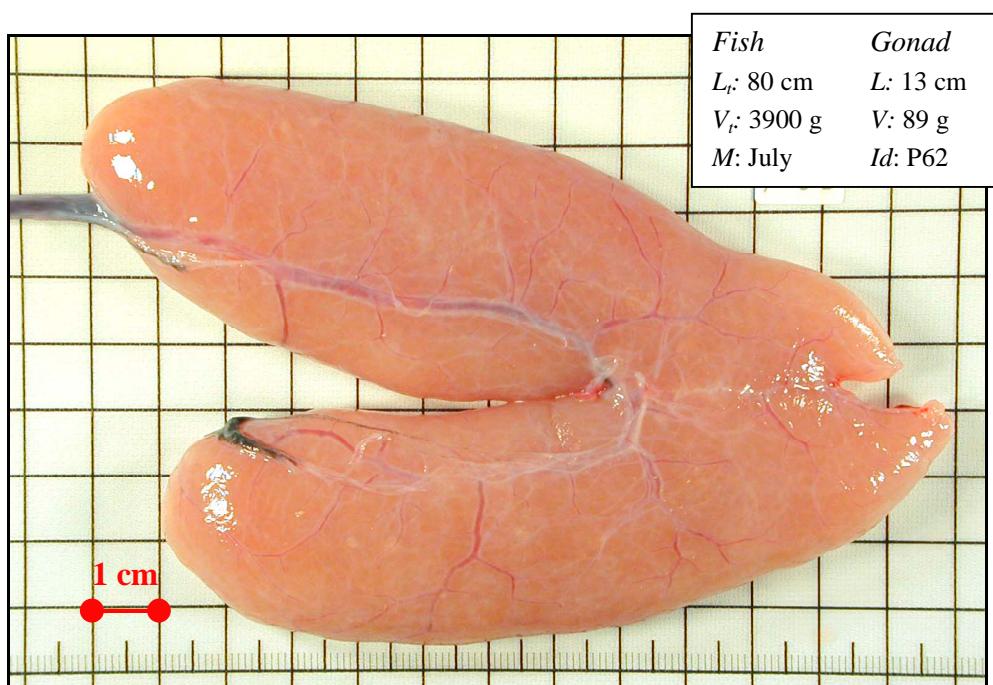


VIII. Regeneration 1: Spent

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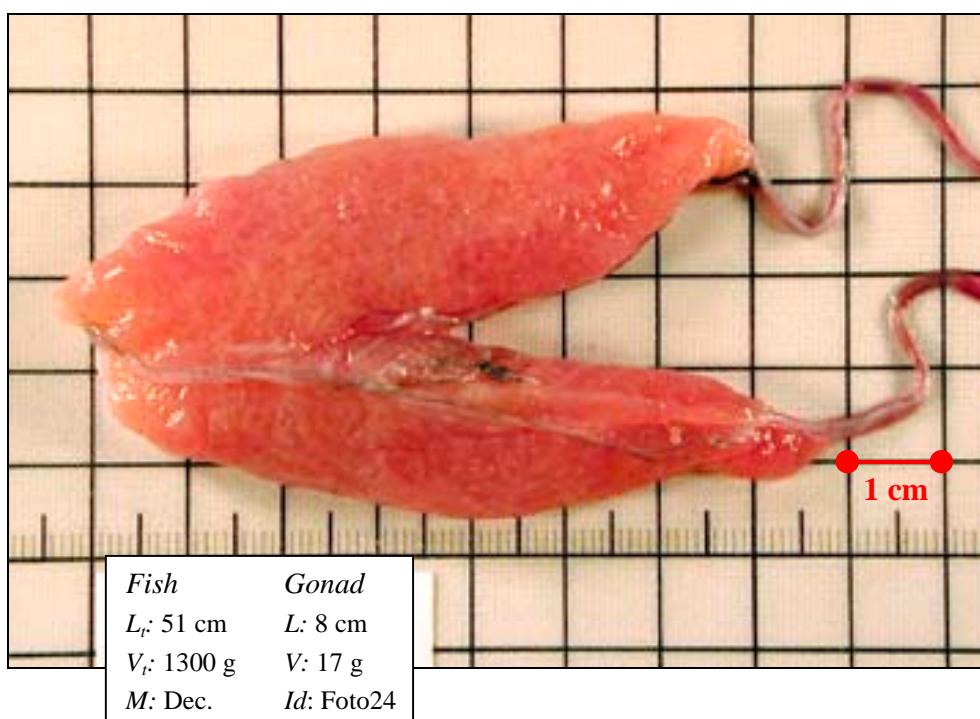
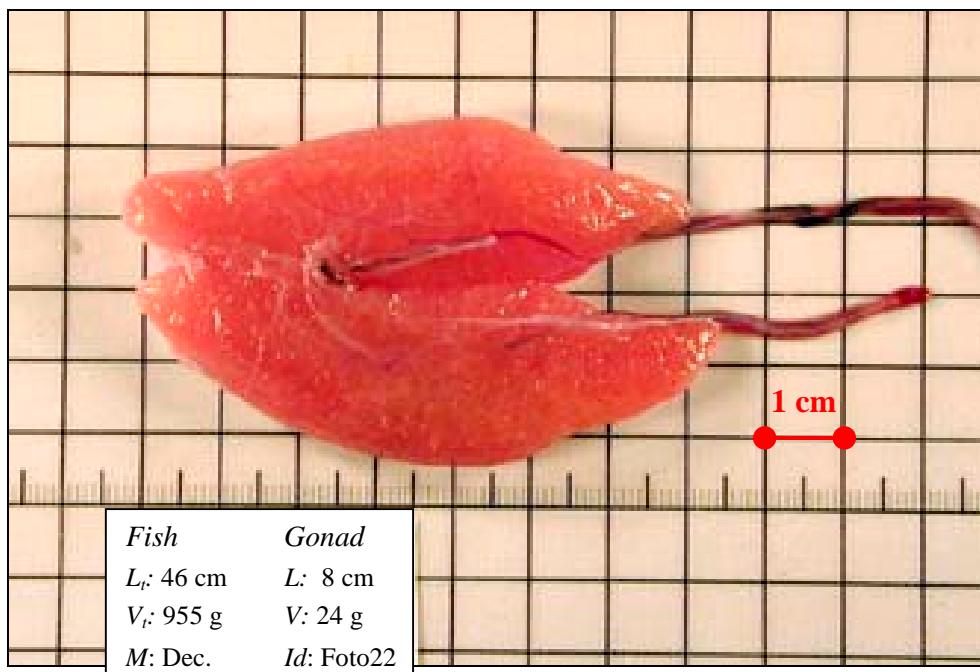


VIII



IX. Regeneration 2: Resting and spawning omission

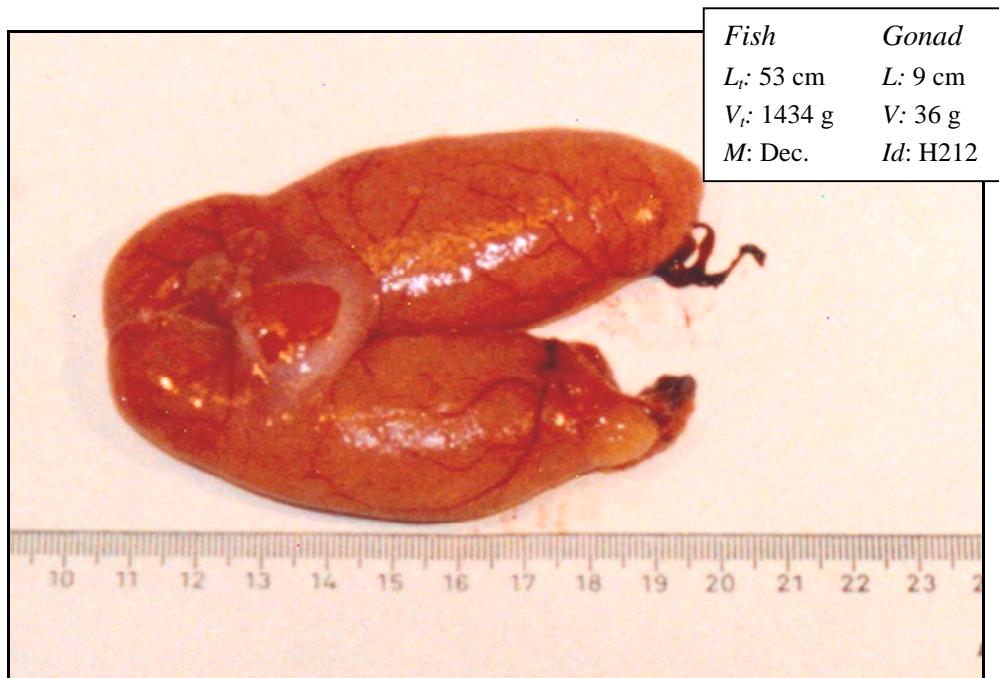
Ovaries small as in II, but with signs of previous spawning; e.g. greyish cast and somewhat uneven walls; blurred translucent, reddish-grey, but more granulated and opaque than in II. Spawning omission considers specimens in Stage IX in February-July. GSI: 1-3.



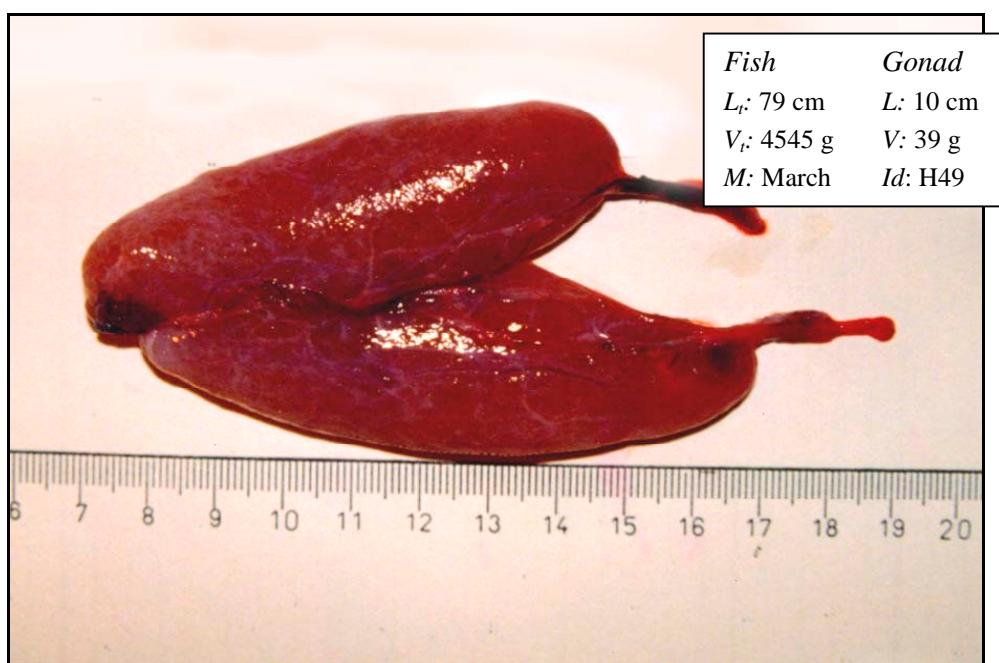
IX. Regeneration 2: Resting and spawning omission

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Tip! Ovaries of larger females often have a greyish cast hampering separation of Stage III and IX. The orange shine indicating yolk formation (Stage III) is easier seen, if the ovary is cut open. H49: spawning omission.

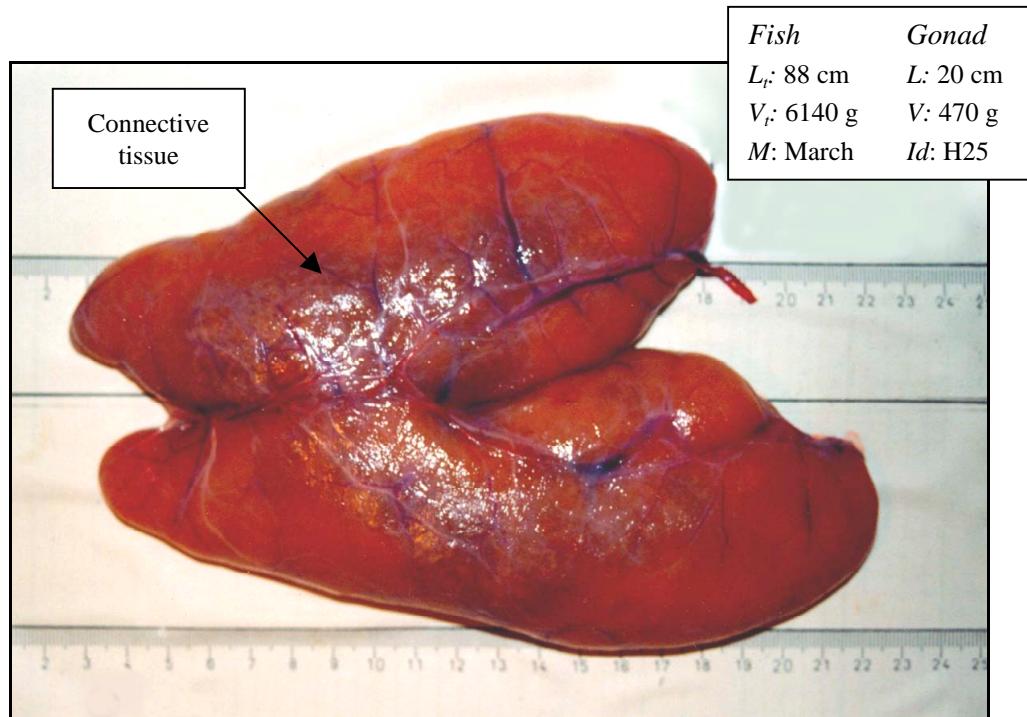
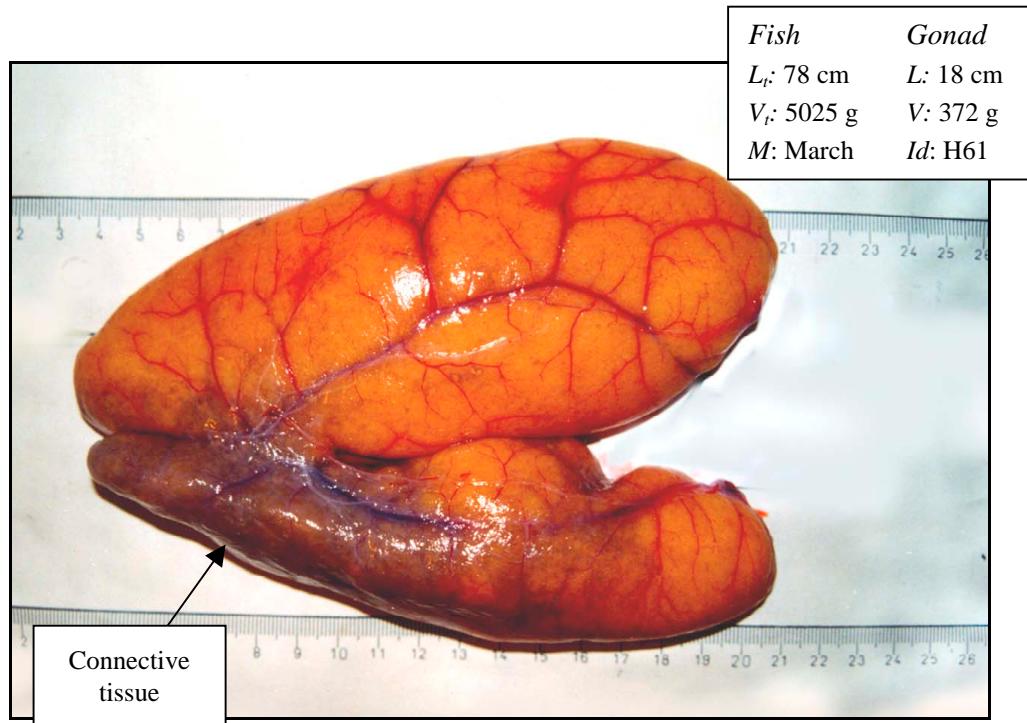


IX



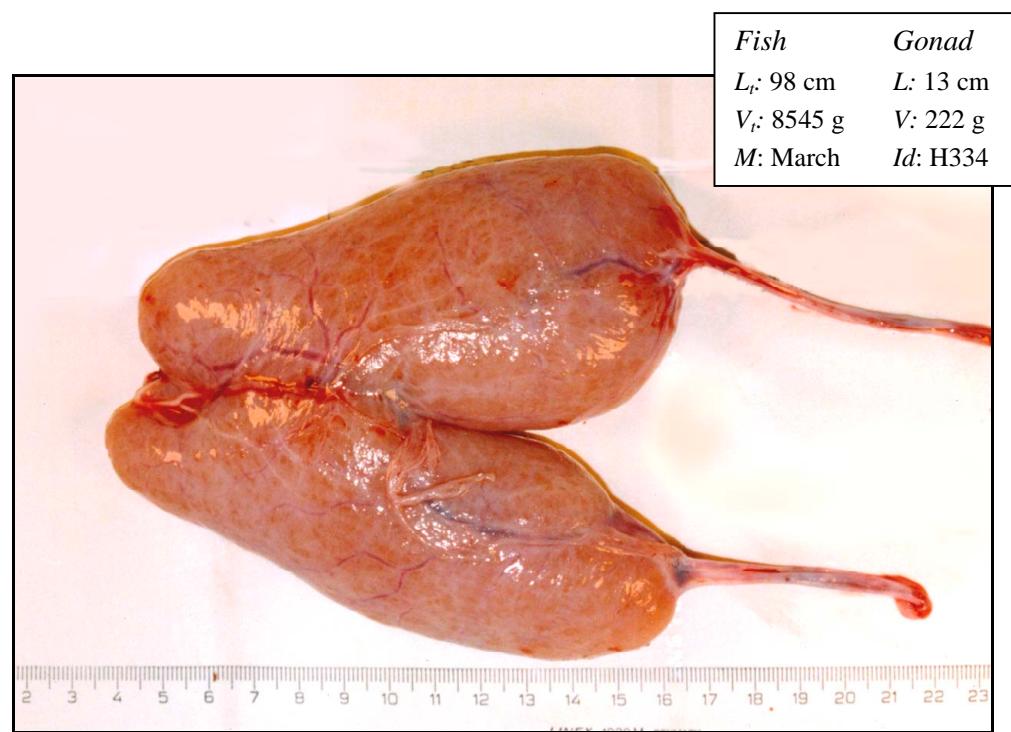
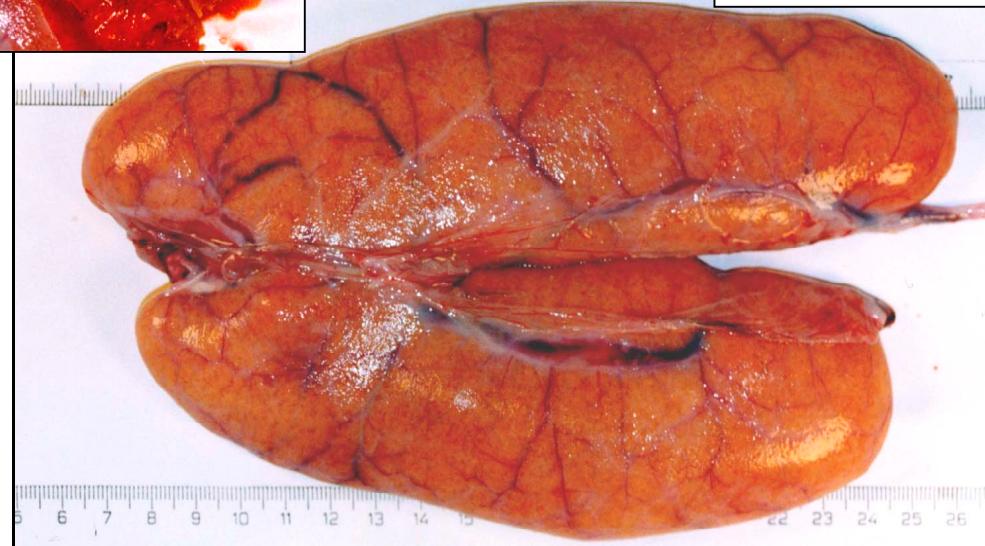
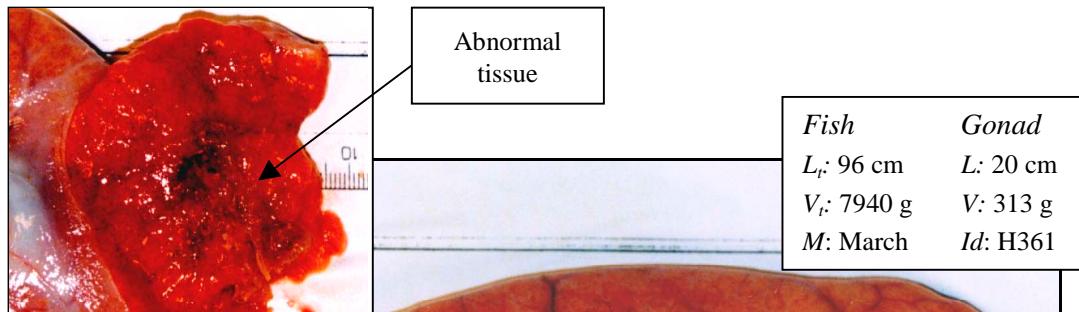
X. Degeneration: Reduced fertility

A: Ovaries with fibrous tissue formation; affected areas compact and hard, brownish-yellow opaque; non-affected parts with normal development. Observed in females from 65 cm. B: Other abnormalities.



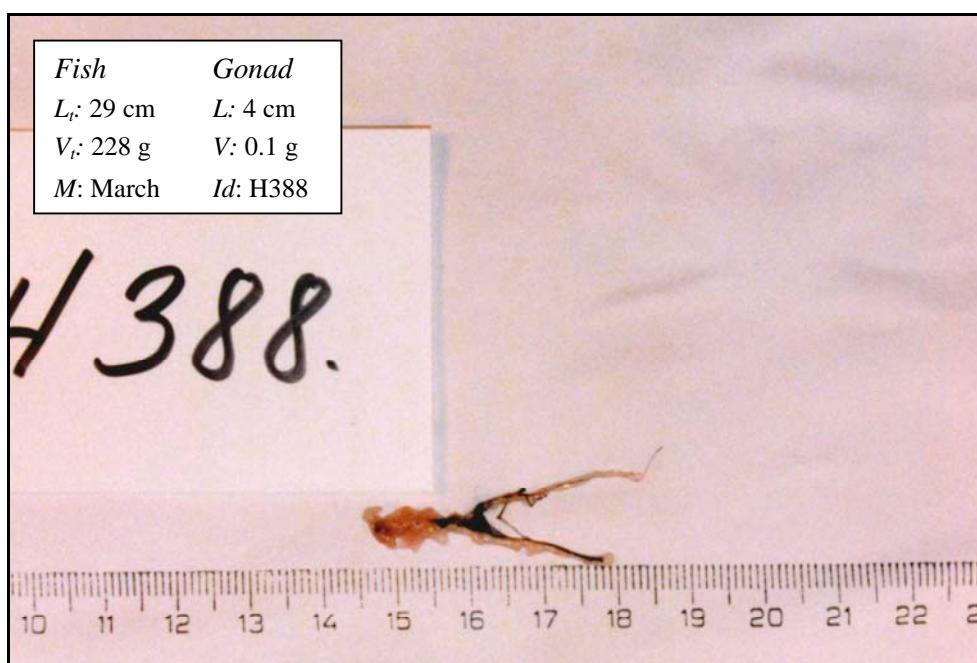
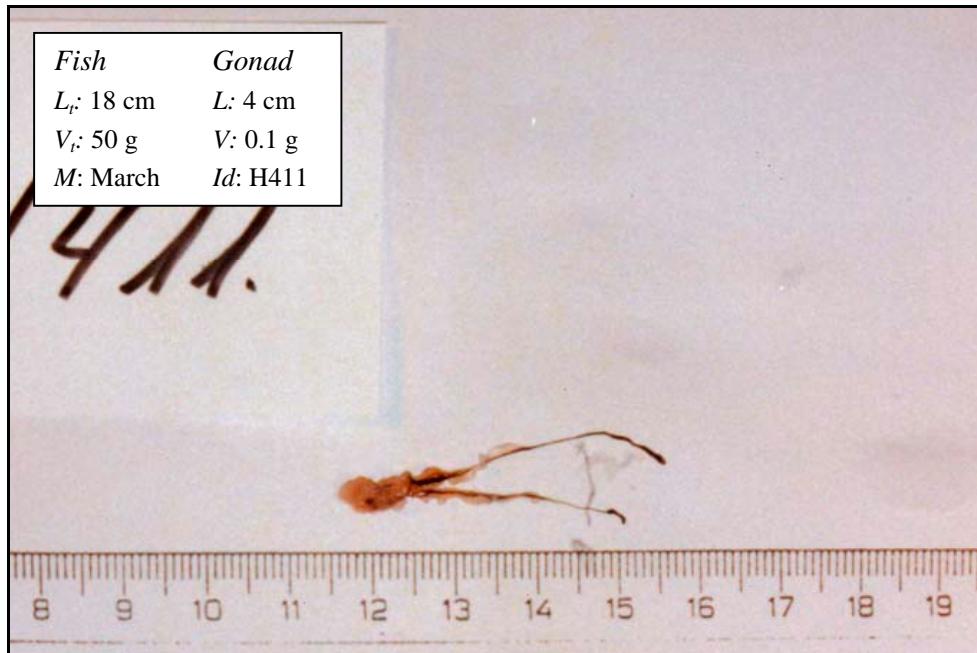
X. Degeneration: Reduced fertility

(continued)



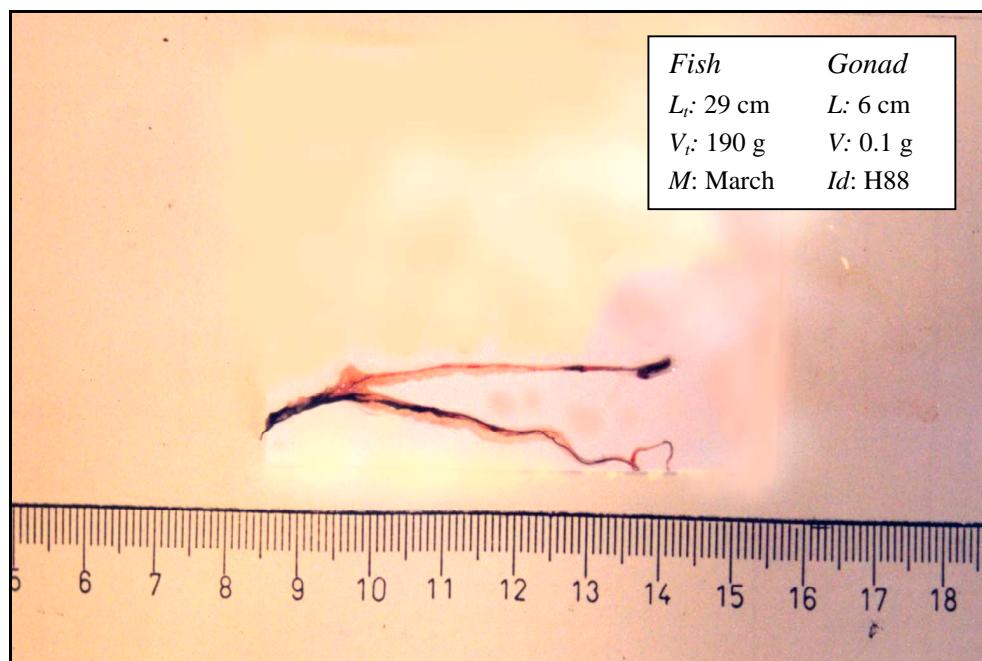
I. Juvenile

Testes emerge as a pair of thin strings along air bladder. Lobules tiny, glassy transparent to reddish translucent in larger specimens. L_T rarely above 30 cm; $GSI < 0.1$.

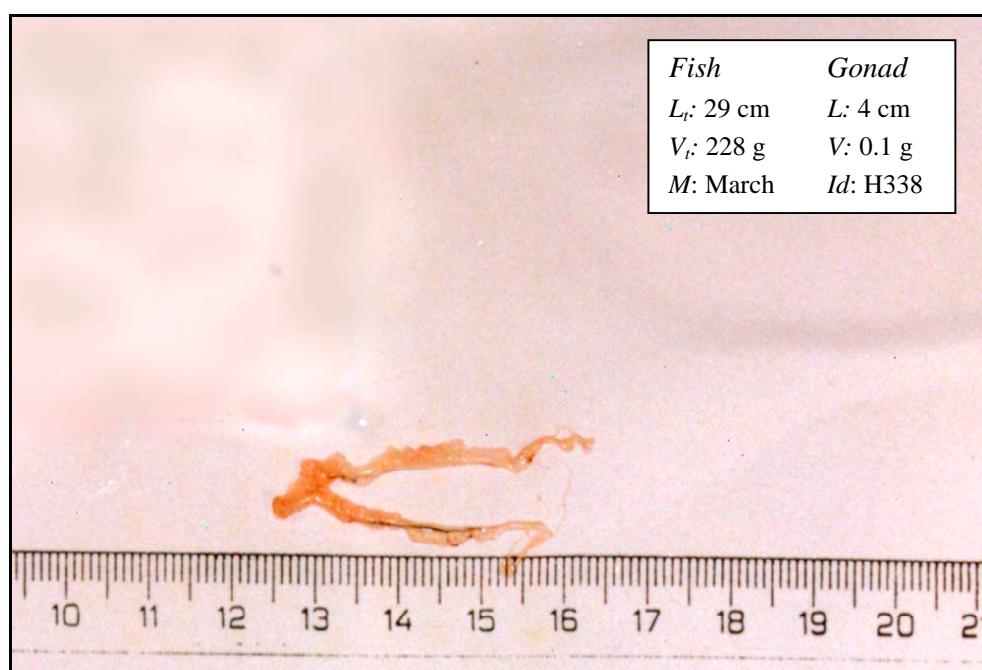


I. Juvenile

(continued)

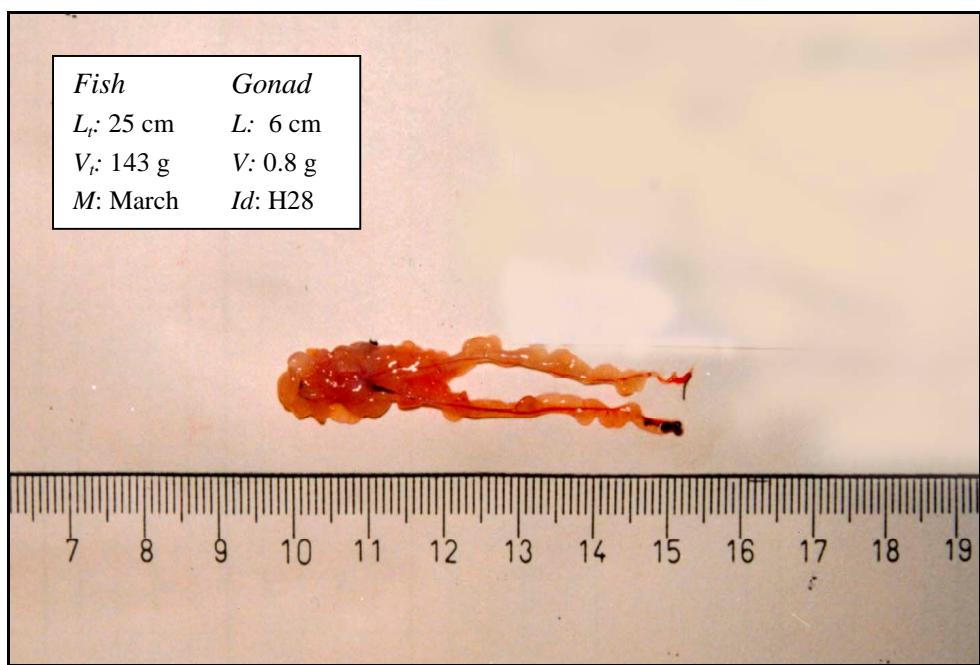
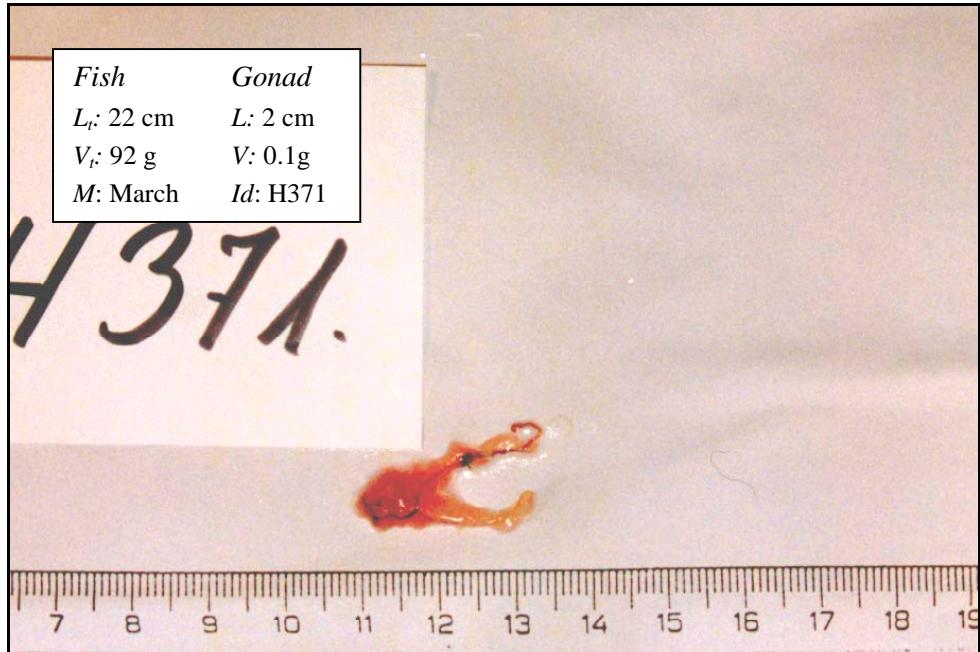


I



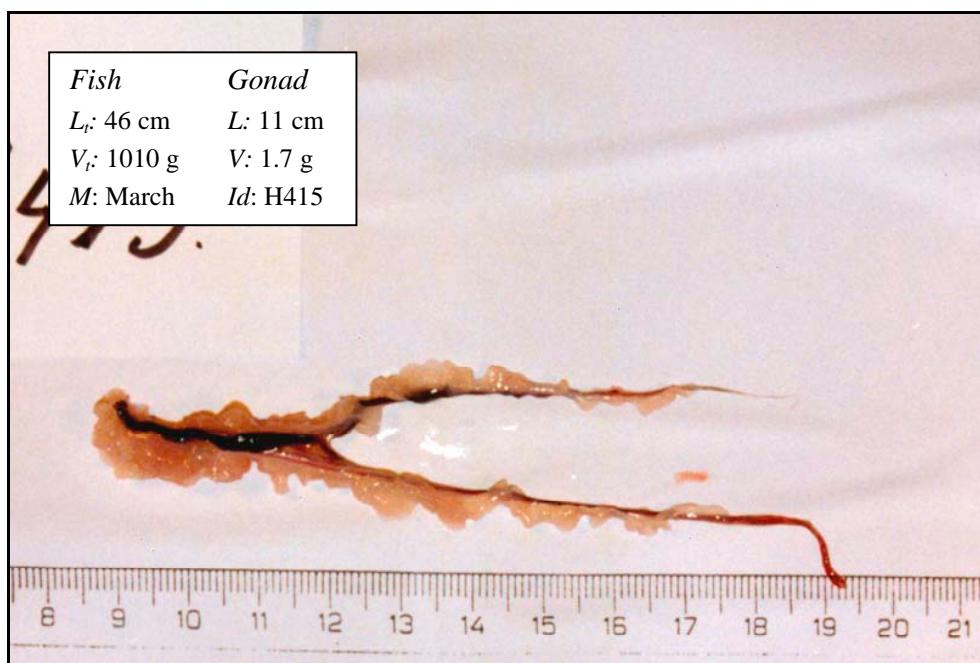
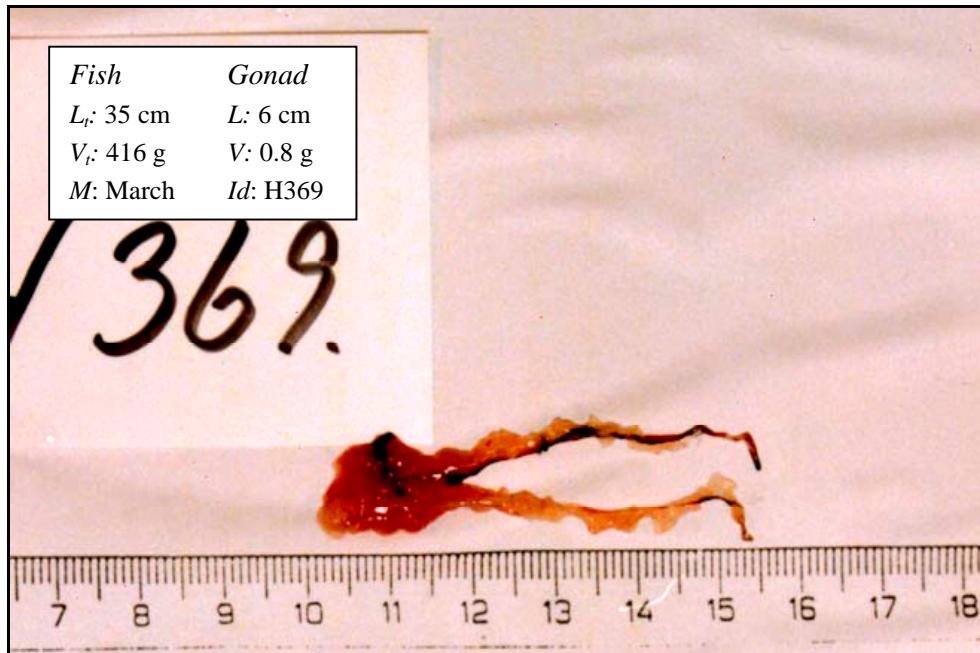
II. Preparation

Testes small, but distinguishable along air bladder. Lobules small, blurred translucent and reddish. L_T : 20-50cm; GSI: 0.1-0.5.



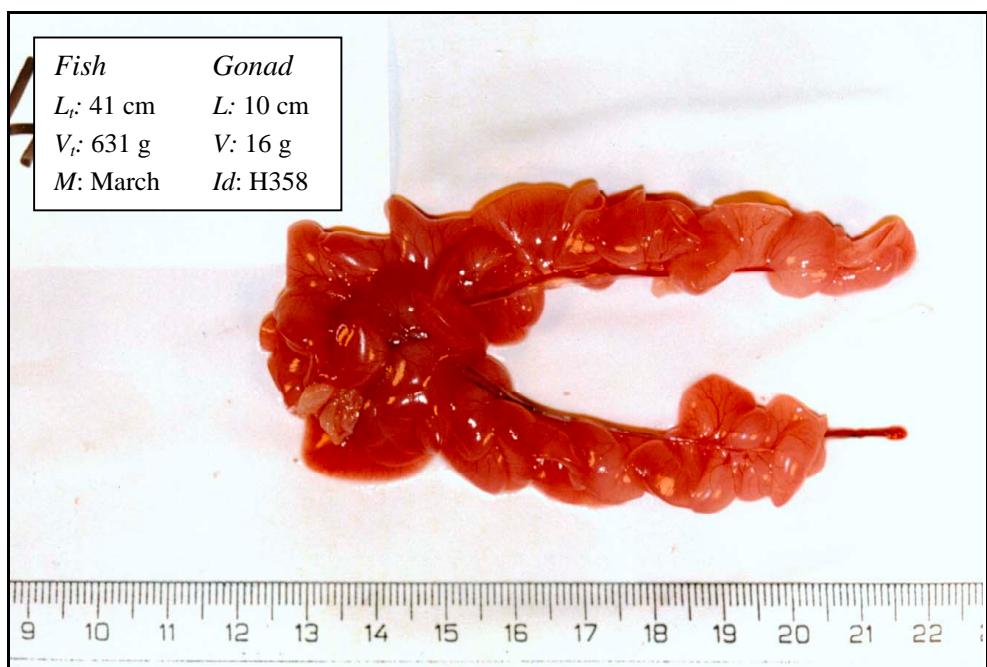
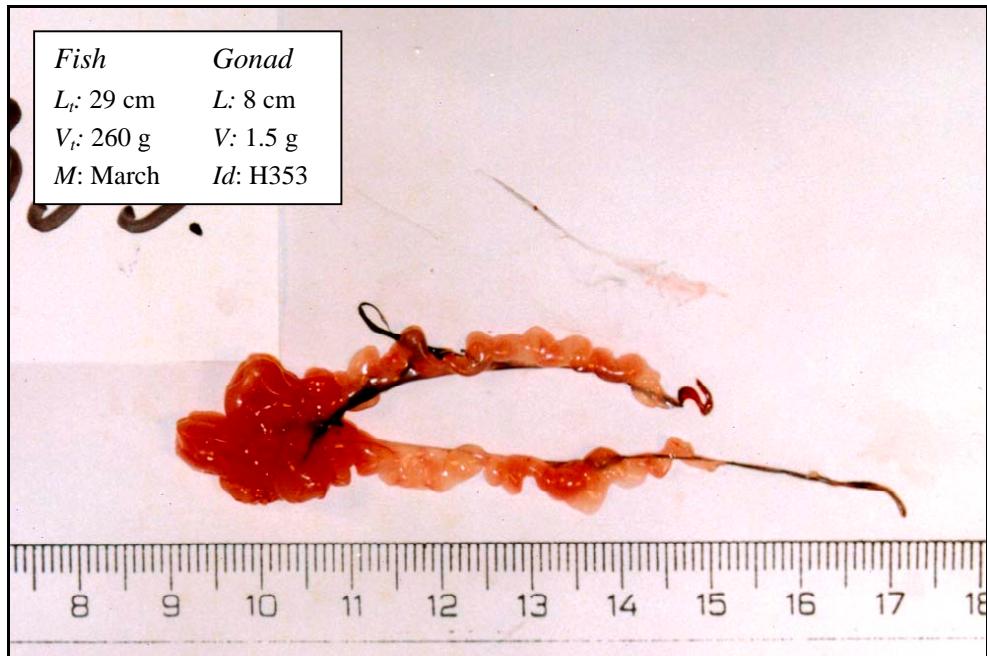
II. Preparation

(continued)



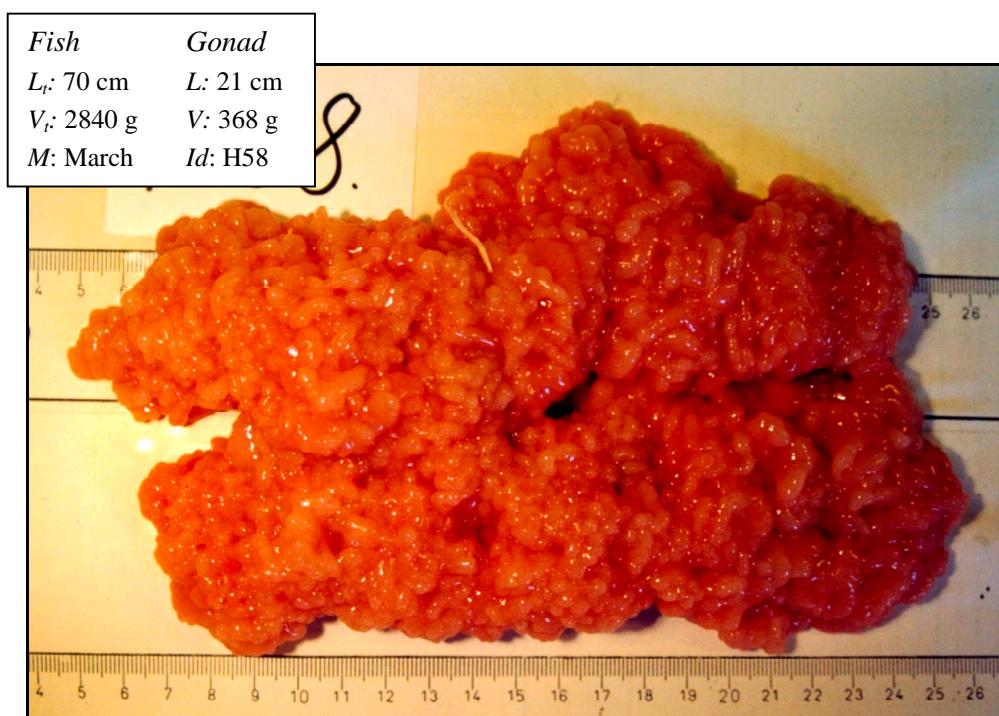
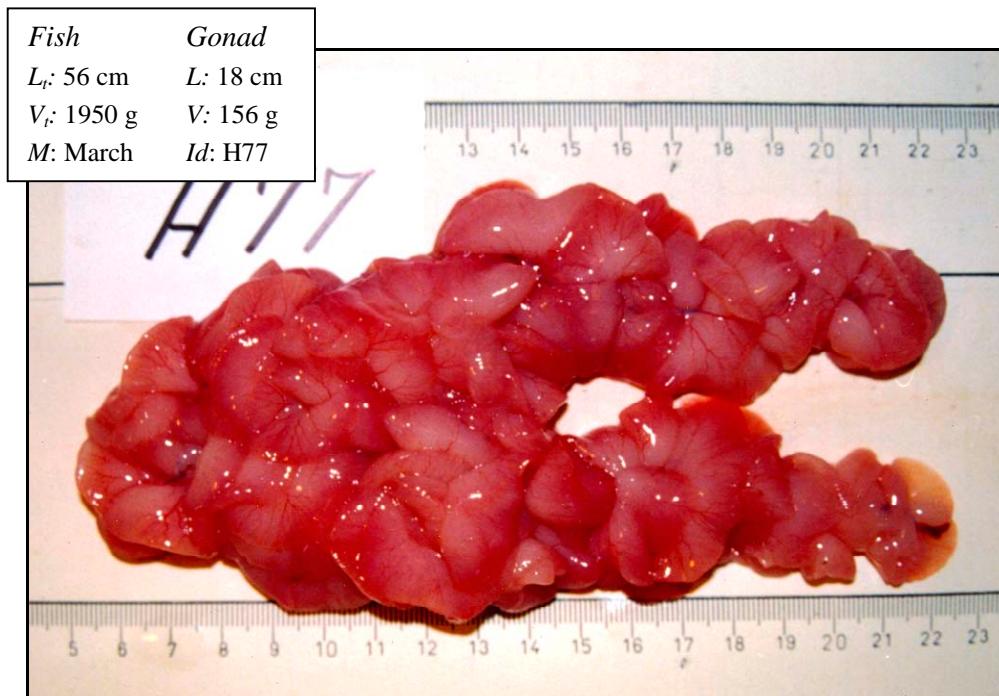
III. Ripening 1: Early spermatogenesis

Testes still small, close to air bladder. Lobules plump and soft, rich in blood vessels, completely or partially opaque, reddish. L_T rarely below 20 cm; GSI: 0.5-6.



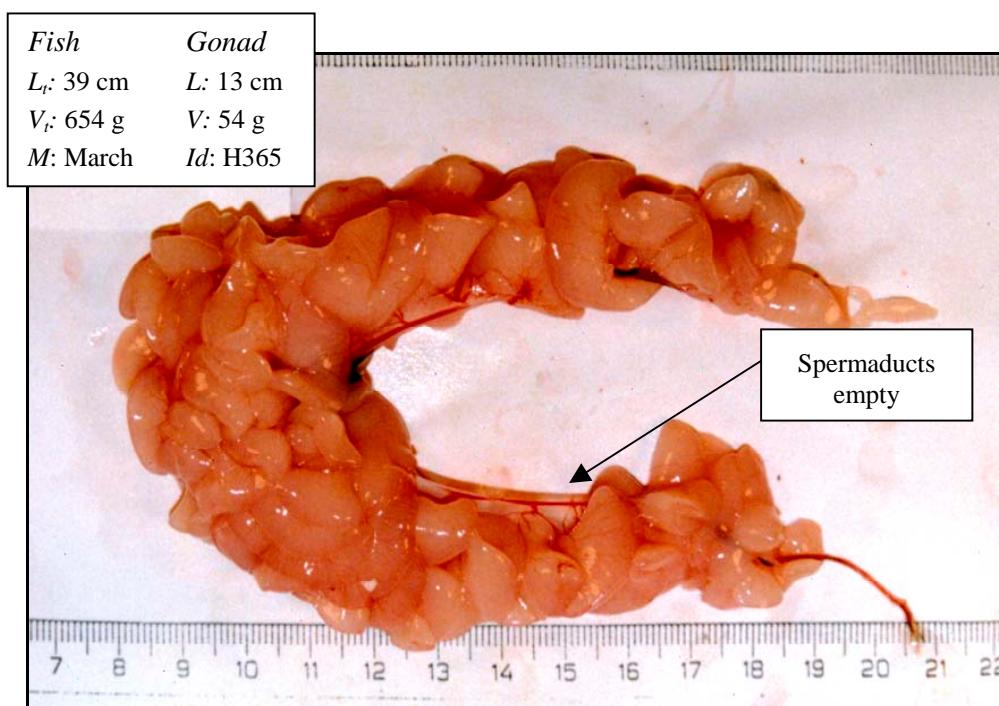
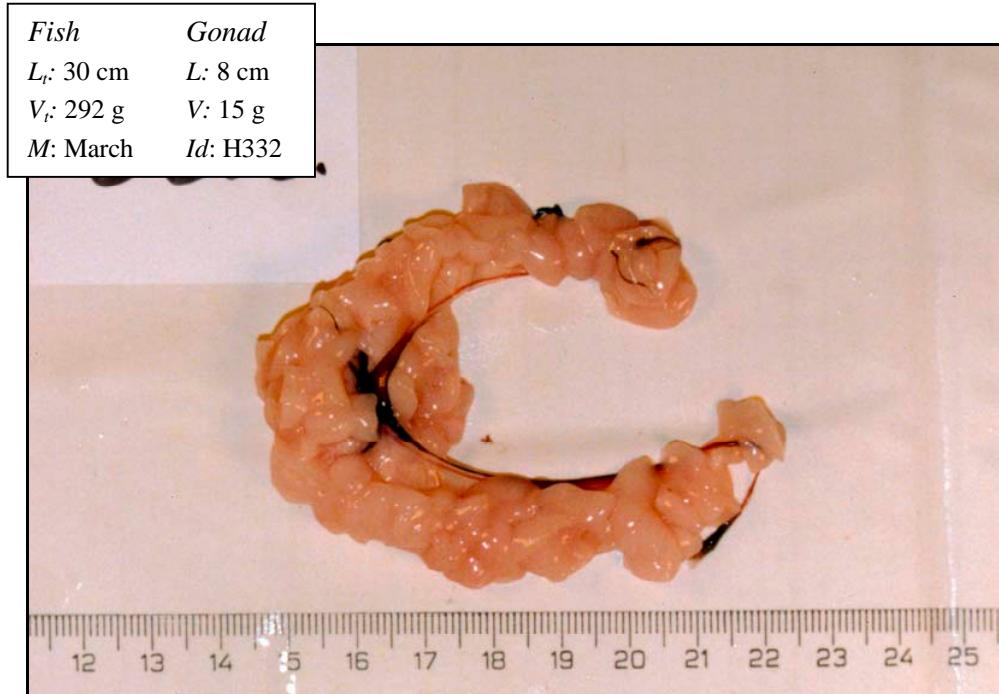
III. Ripening 1: Early spermatogenesis

(continued)



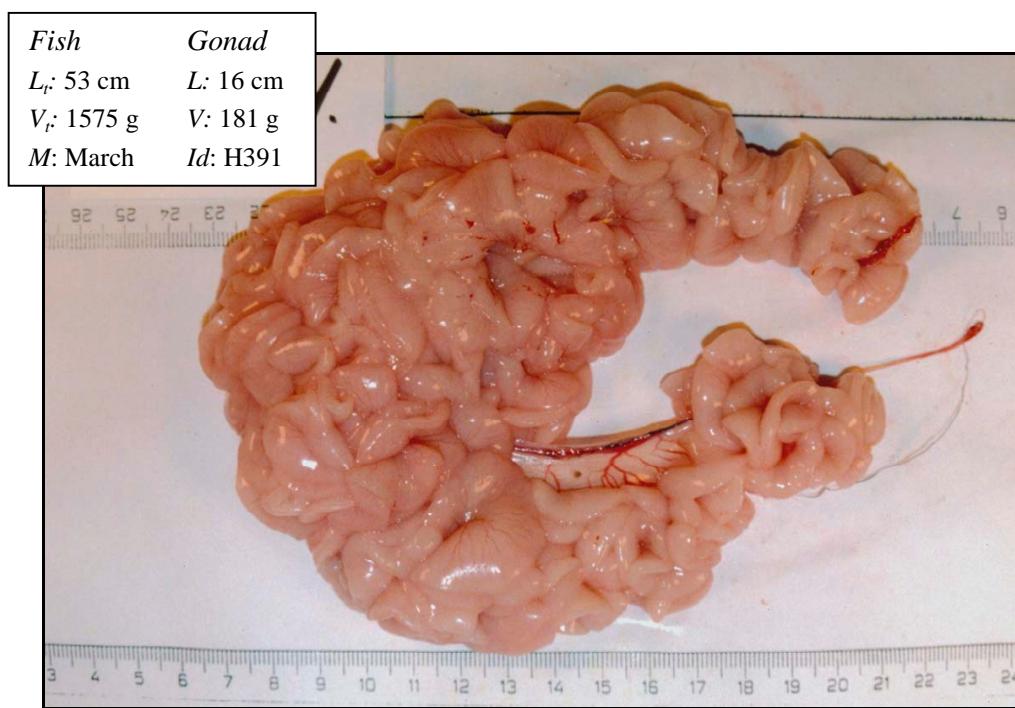
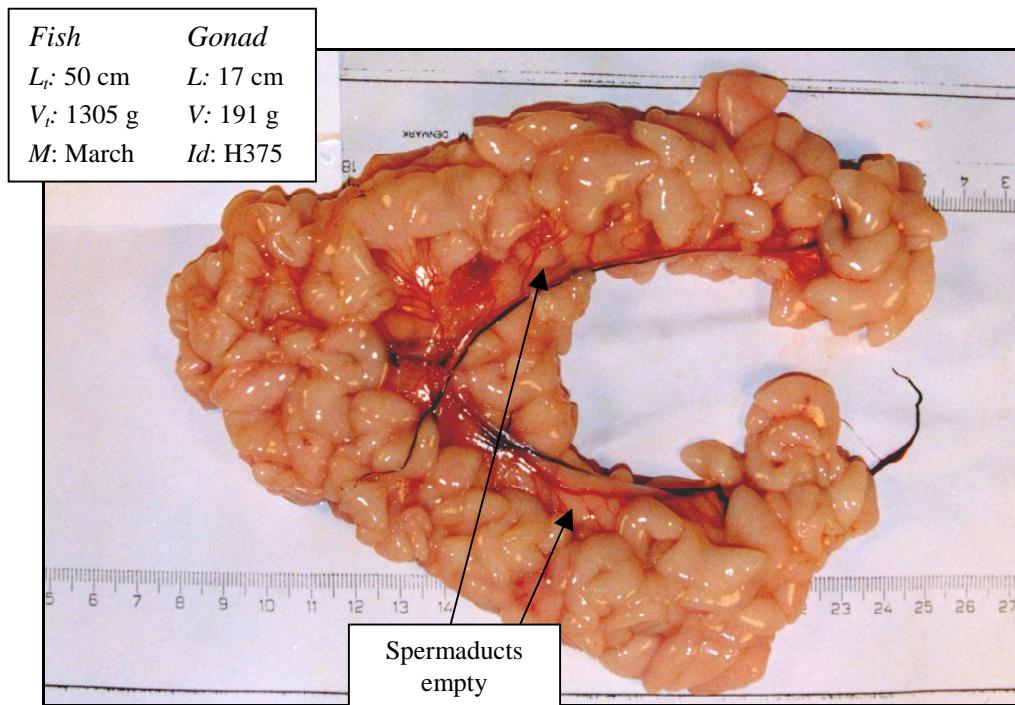
IV. Ripening 2: Late spermatogenesis

Testes enlarged and prominent dorsal in body cavity; Lobules plump and brittle; reddish-white. Empty, transparent spermatducts with prominent blood vessels; no sperm release. *GSI*: 1-18.



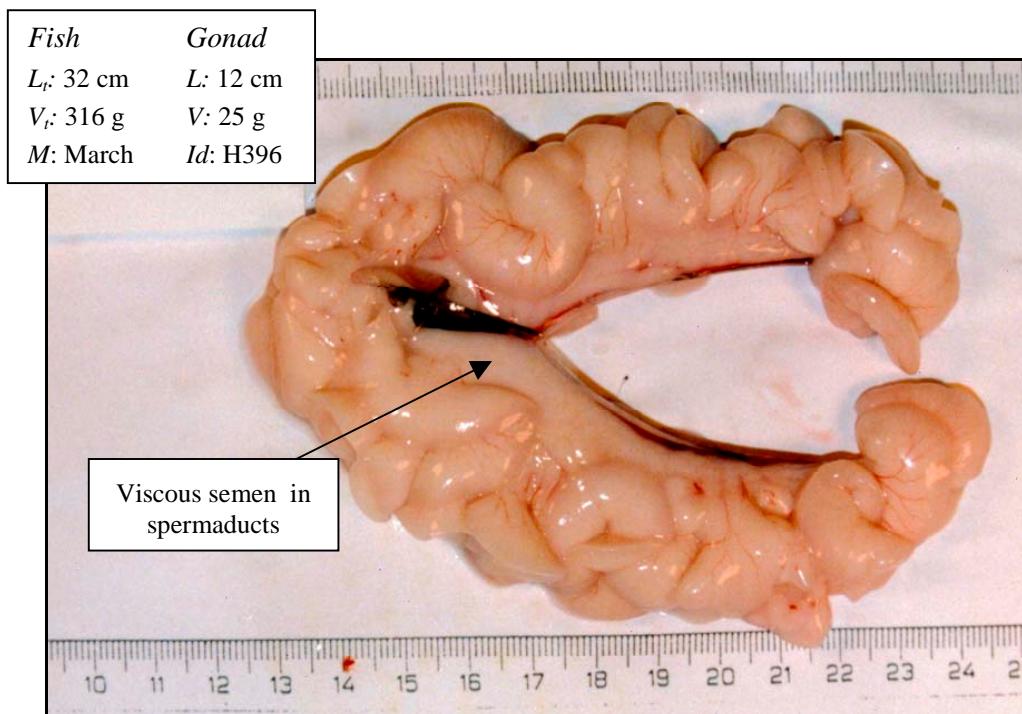
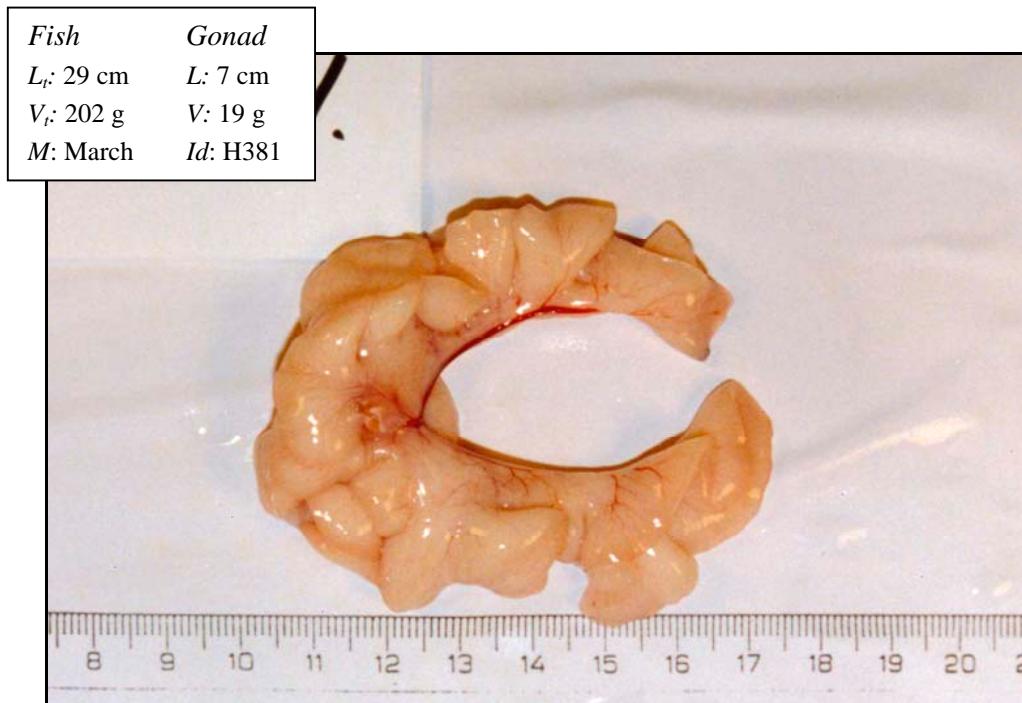
IV. Ripening 2: Late spermatogenesis

(continued)



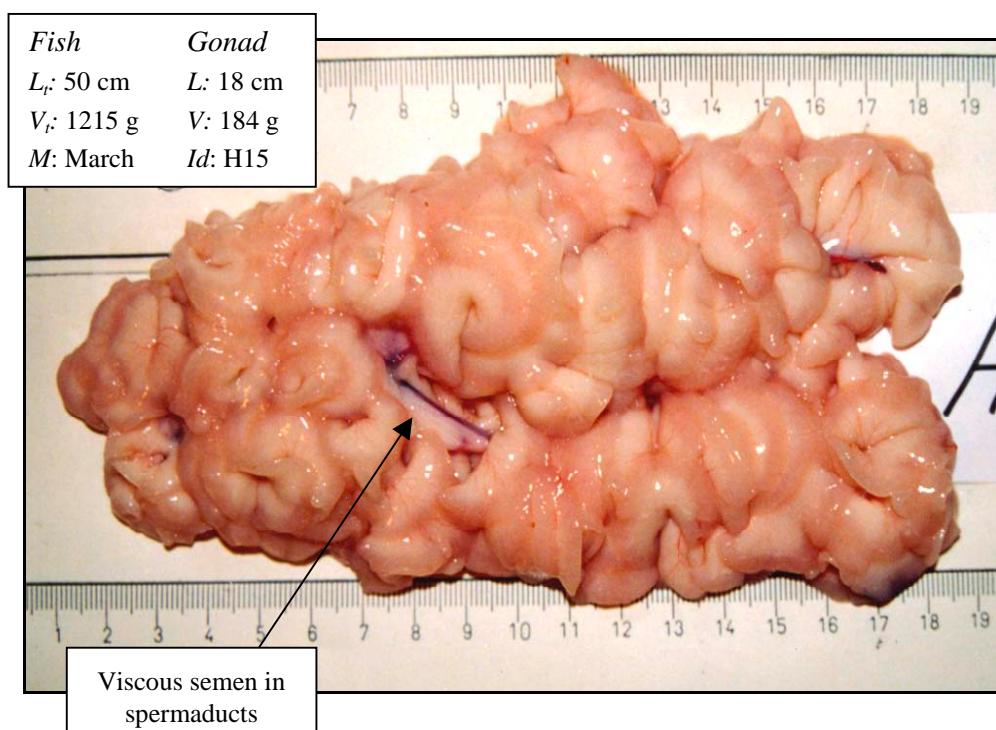
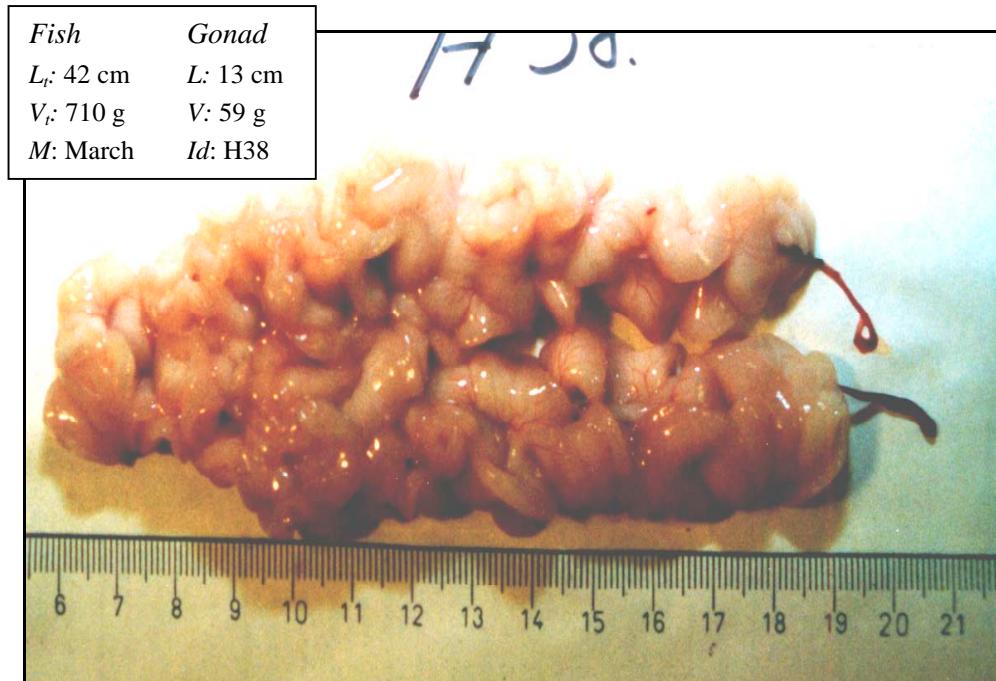
V. Spawning 1: Initiation of spawning

Testes extending into ventral part of body cavity. Lobules distended and brittle, opaque creamy-white. Spermaducts filled with viscous semen and a viscous droplet may be released from vent. *GSI*: 3-22.



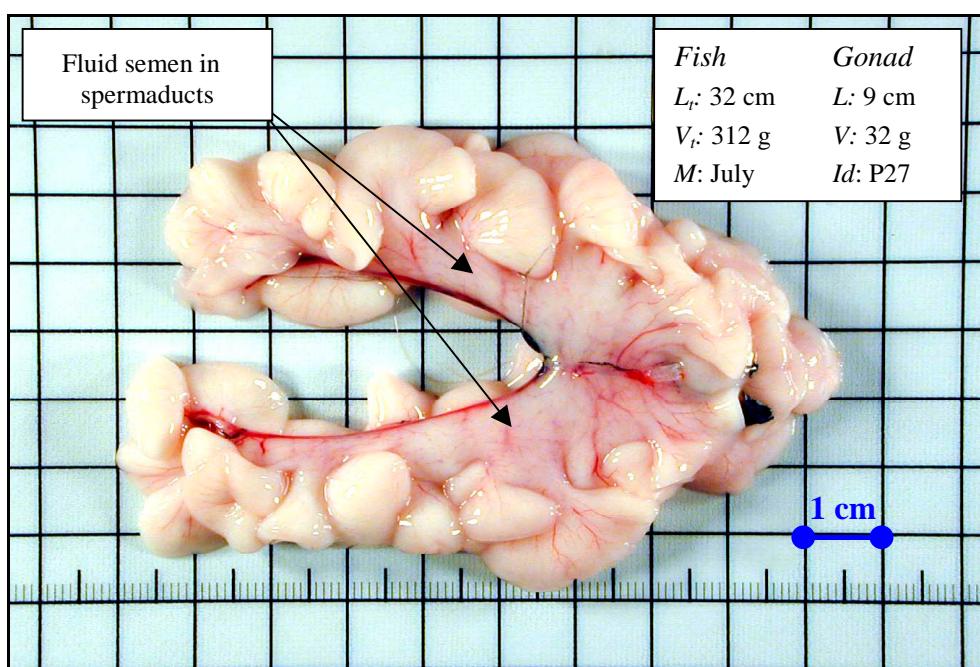
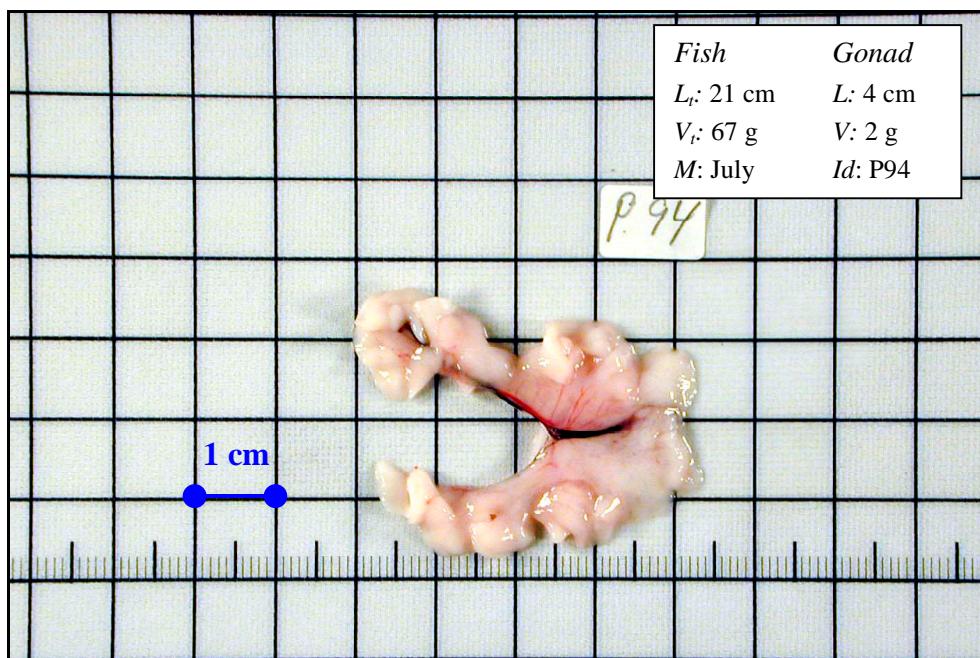
V. Spawning 1: Initiation of spawning

(continued)



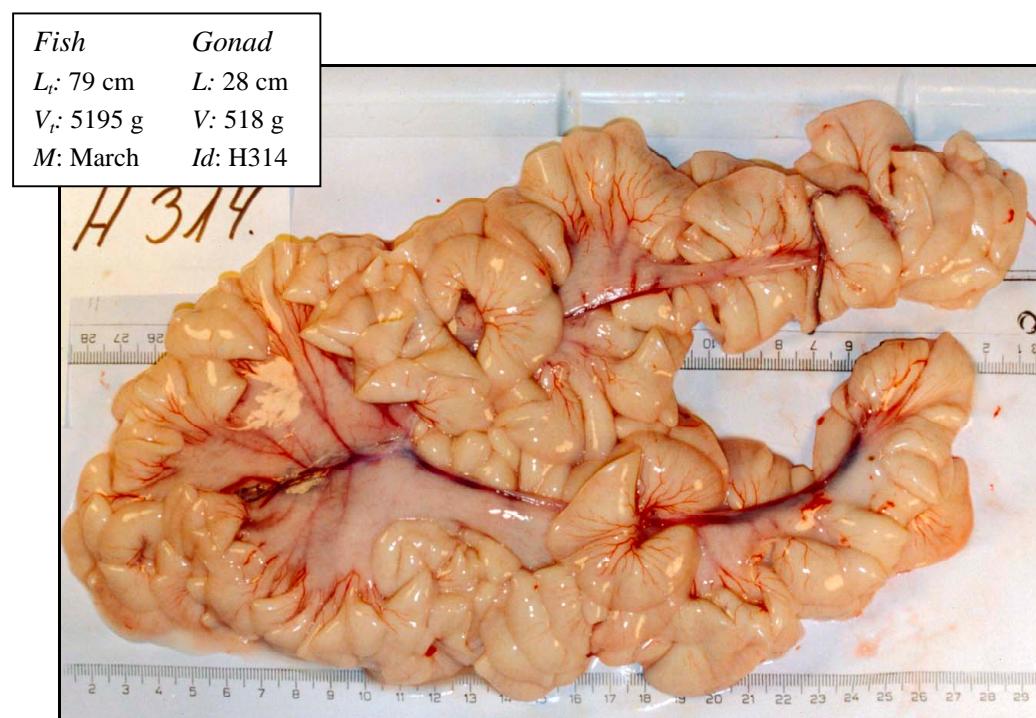
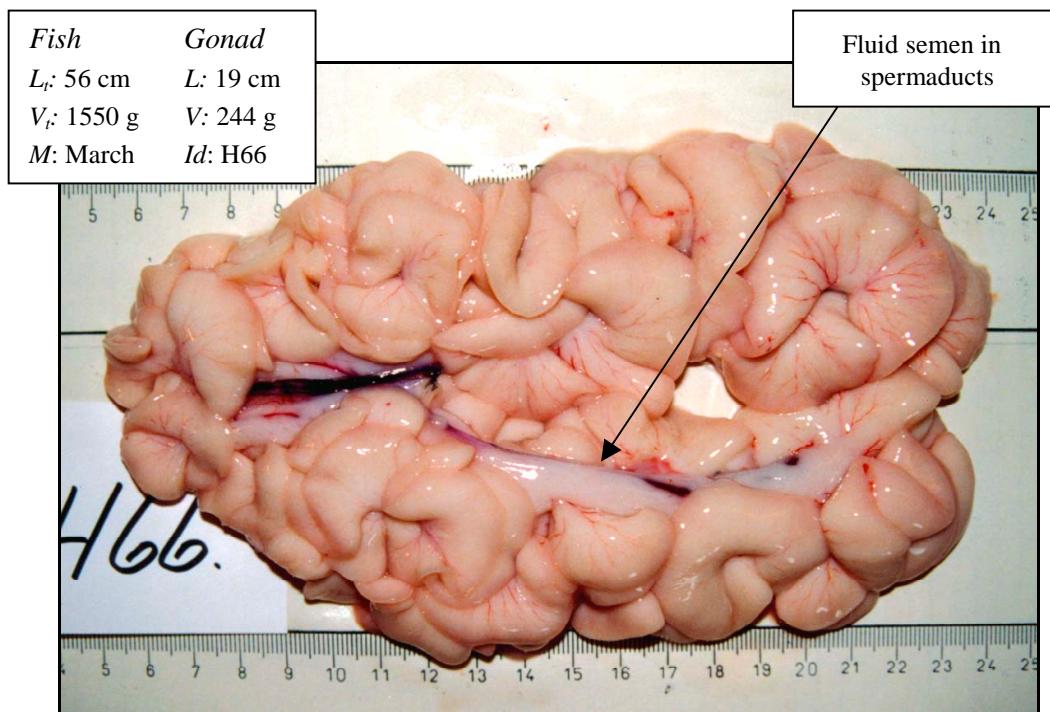
VI. Spawning 2: Main spawning period

Testes large and prominent in body cavity (as in V). Lobules still plump, but soft; completely opaque, whitish. Spermaducts filled with fluid, milky semen that easily flows from vent. GSI: 3 to 25.



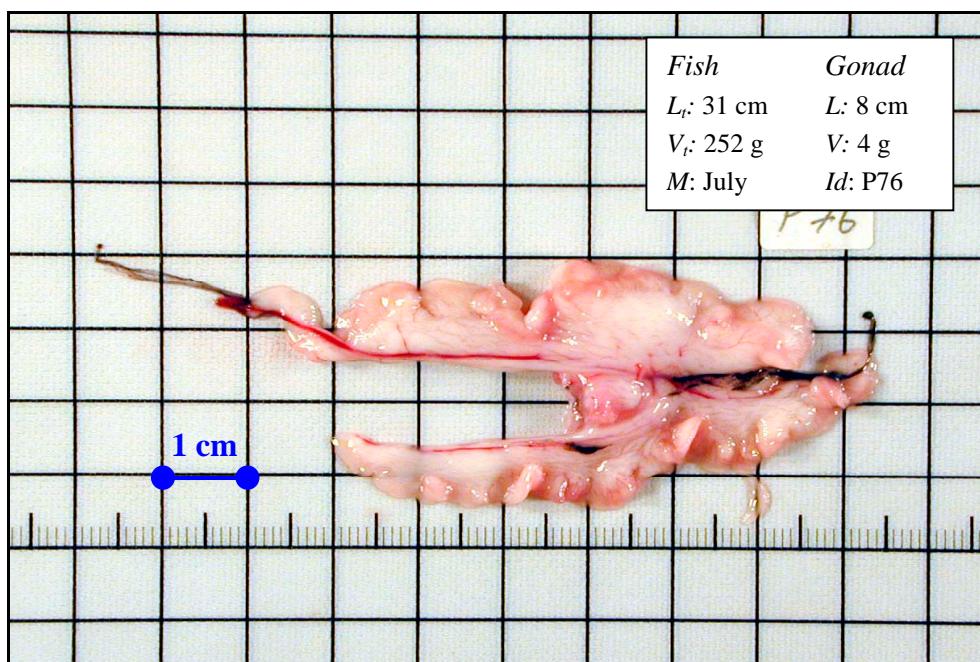
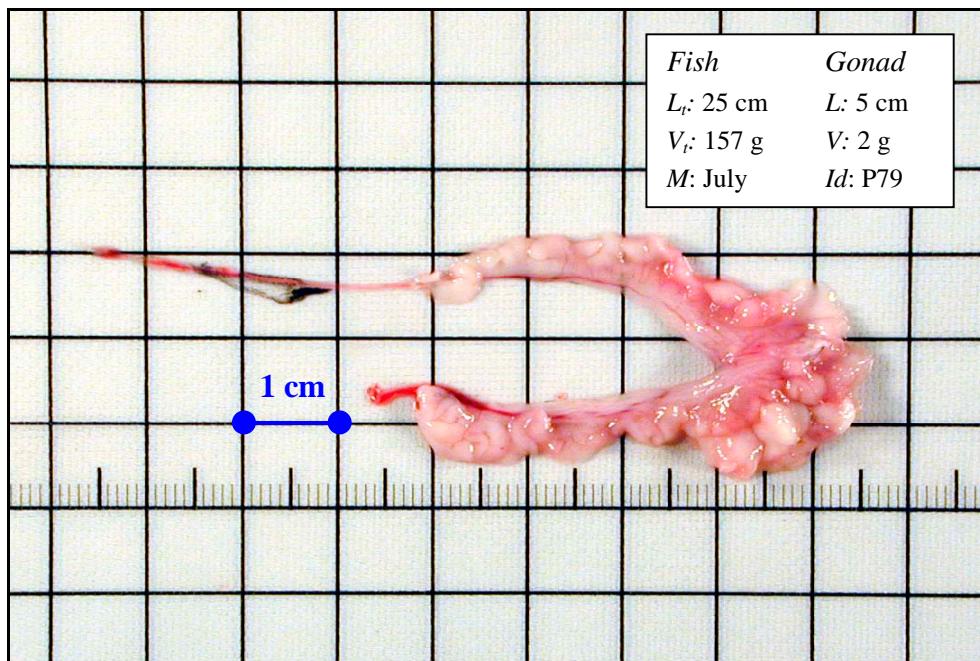
VI. Spawning 2: Main spawning period

(continued)



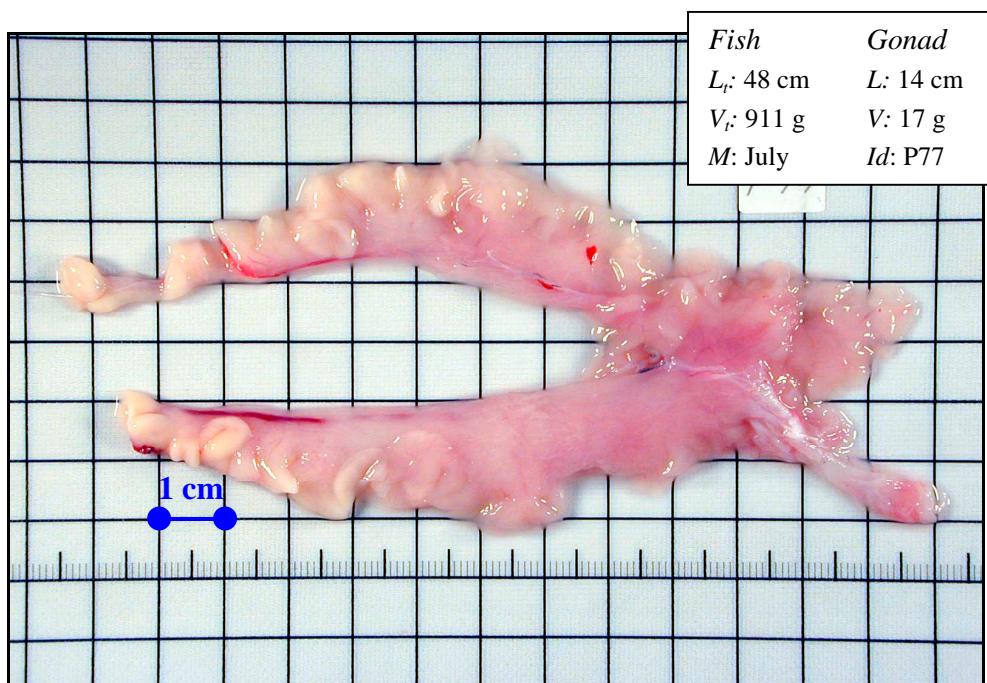
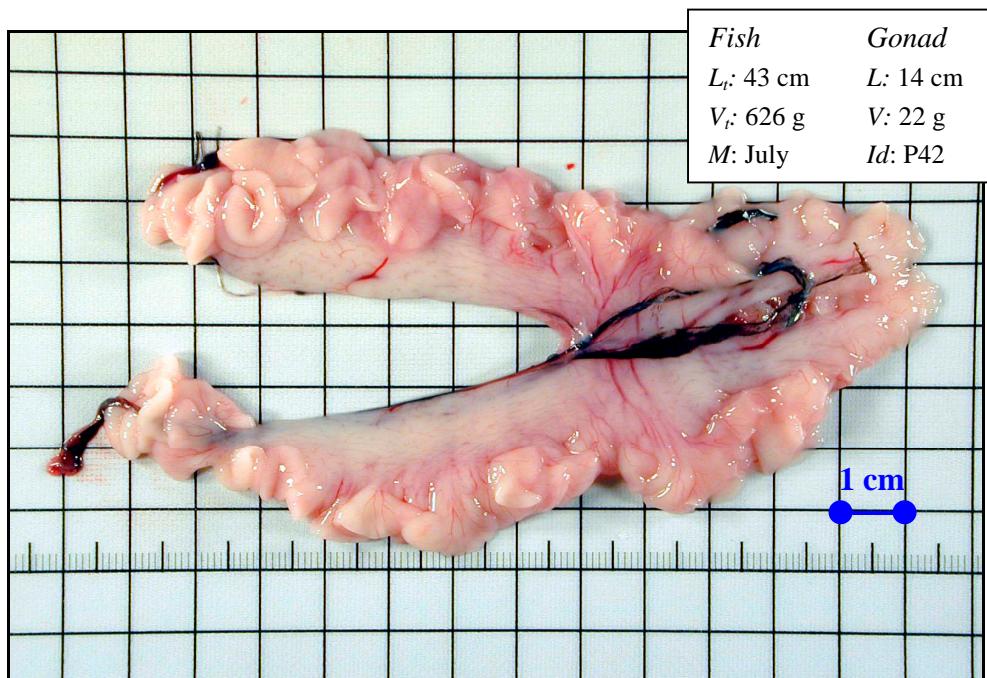
VII. Spawning 3: Cessation of spawning

Testes shrunk to dorsal part of body cavity; soft and flabby. Lobules almost empty, opaque, reddish-white. Spermatducts still with fluid semen that easily flows from vent. *GSI*: 0.5 to 4.



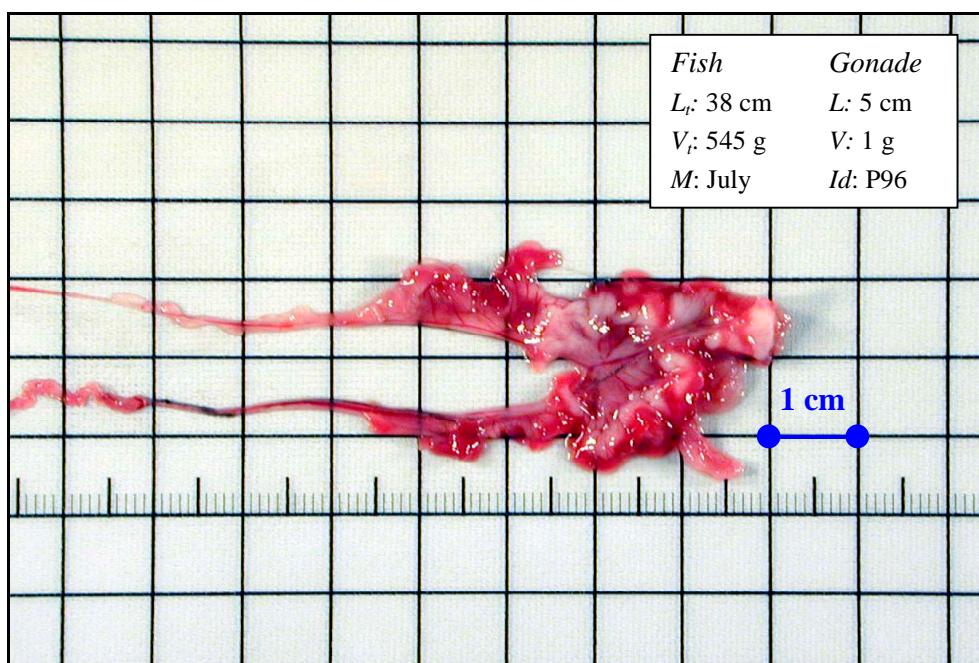
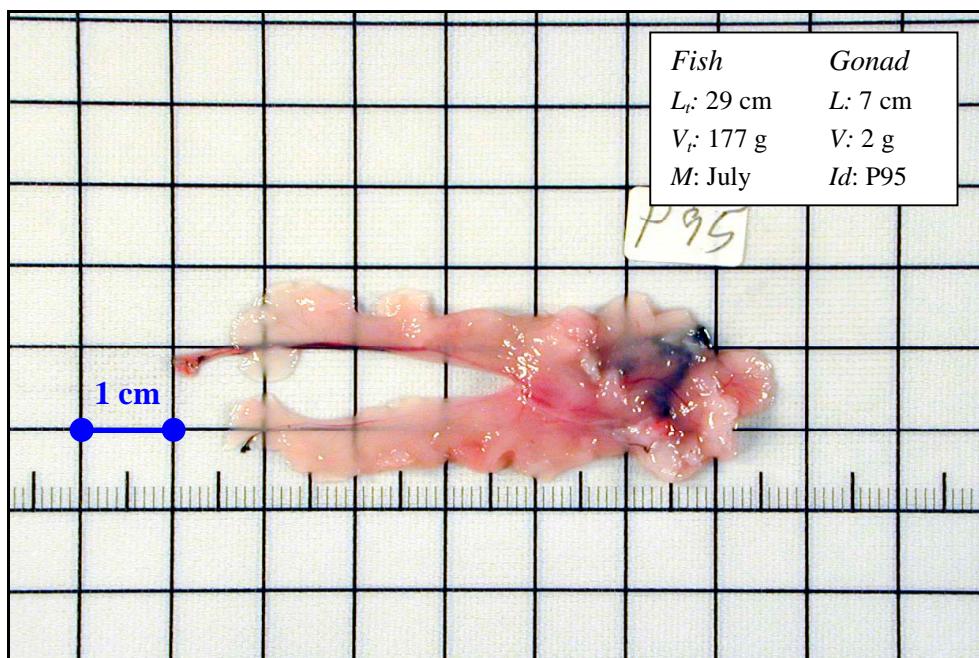
VII. Spawning 3: Cessation of spawning

(continued)



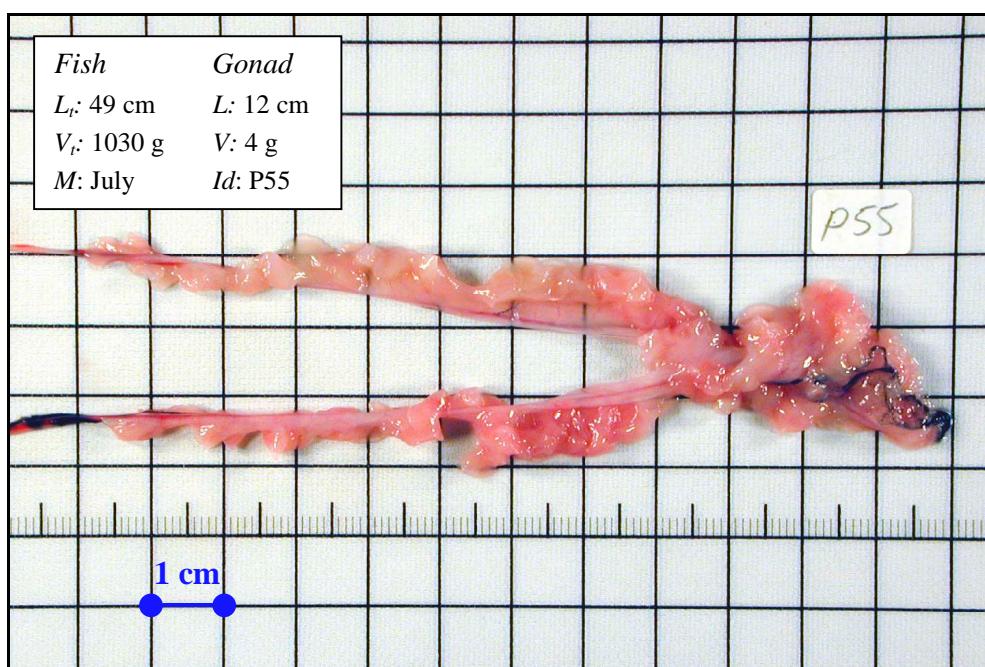
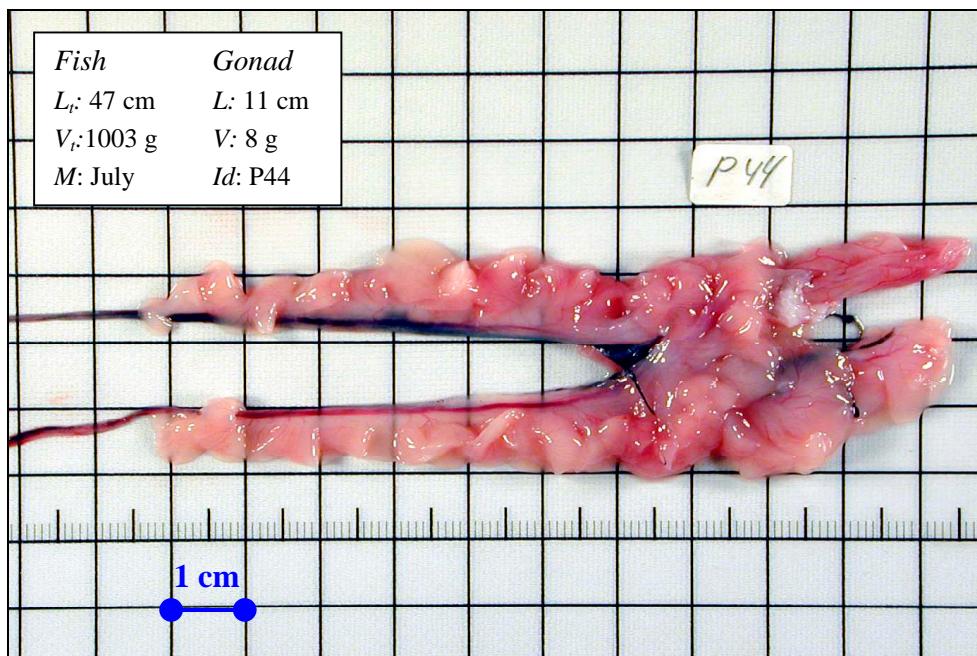
VIII. Regeneration 1: Spent

Testes contracted, close to air bladder; rich in blood vessels. Lobules empty, flabby, reddish potentially with a greyish cast. Spermatducts with signs of previous distension, often with visible remains of semen. $GSI > 1.5$.



VIII. Regeneration 1: Spent

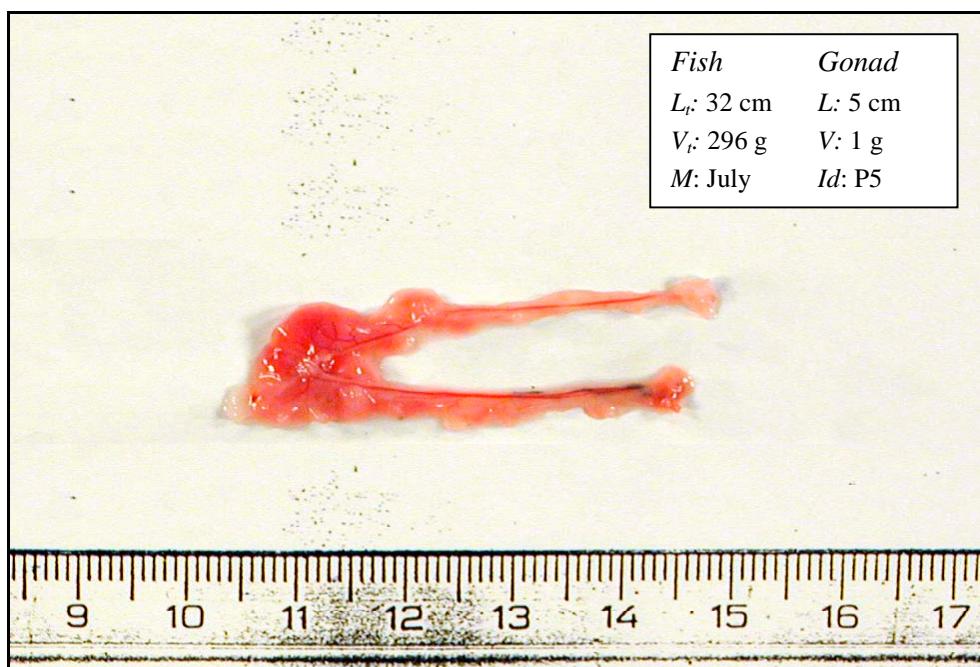
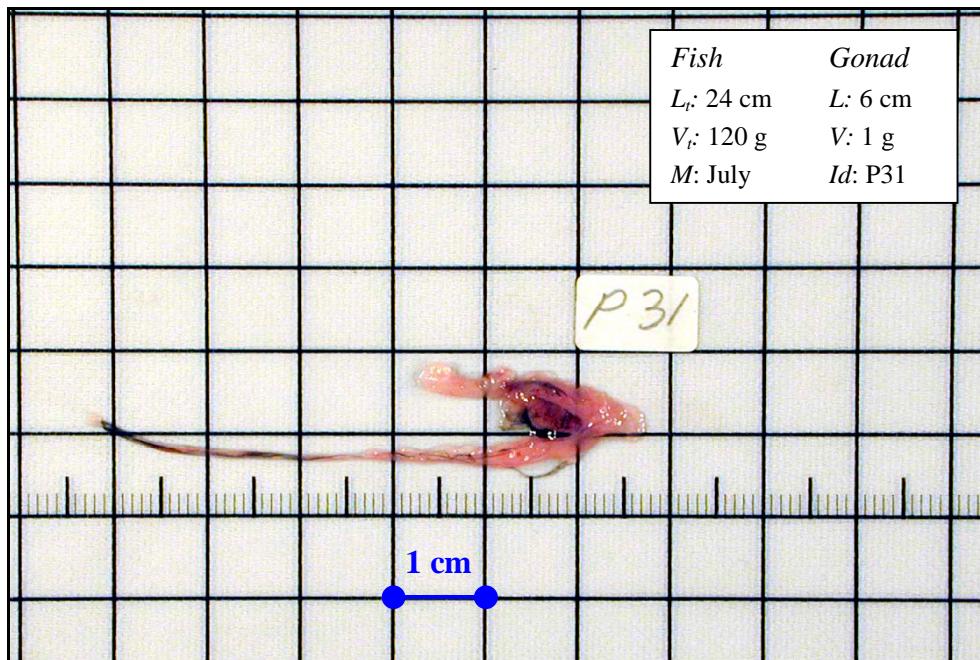
(continued)



VIII

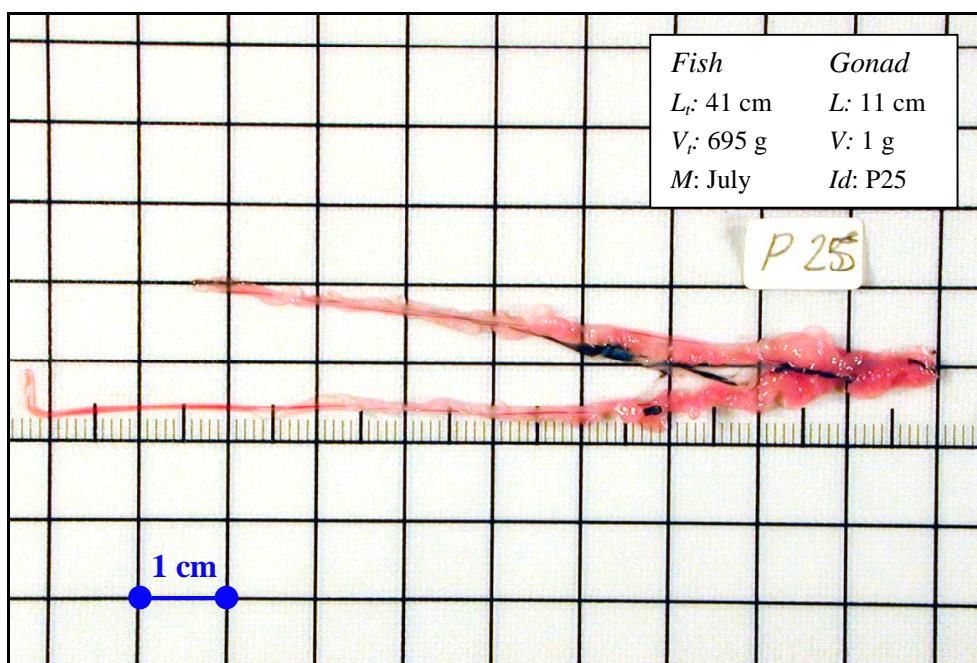
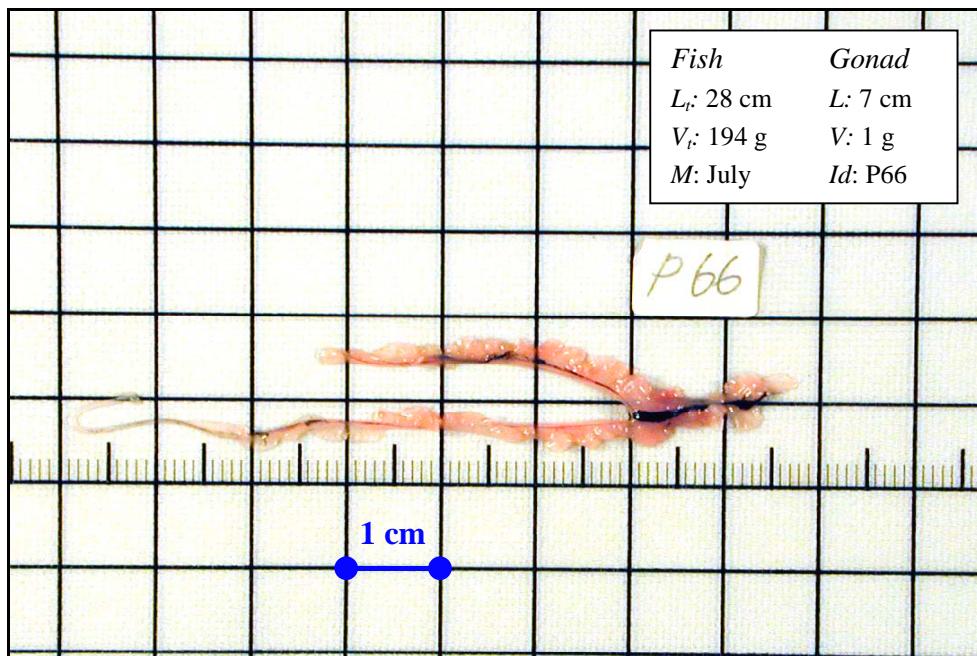
IX. Regeneration 2: Resting and spawning omission

Testes small (as in Stage II), but with signs of previous spawning; e.g. lobules slightly larger than in II; spermaducts often with greyish cast. Spawning omission considers specimens in Stage IX in February-July. *GSI < 1.5.*



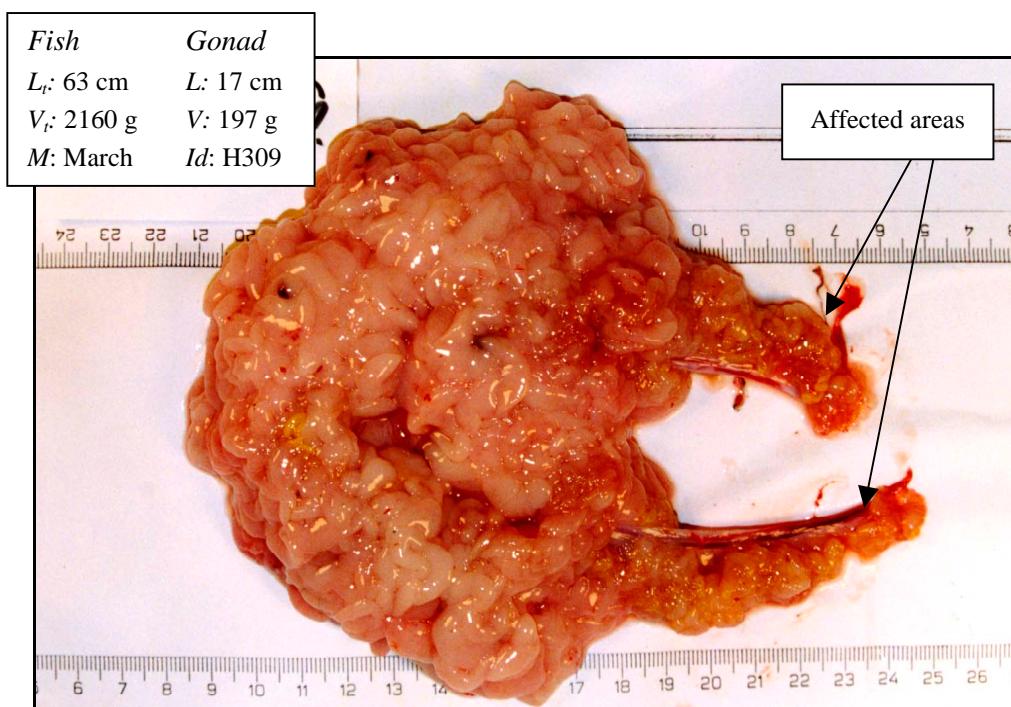
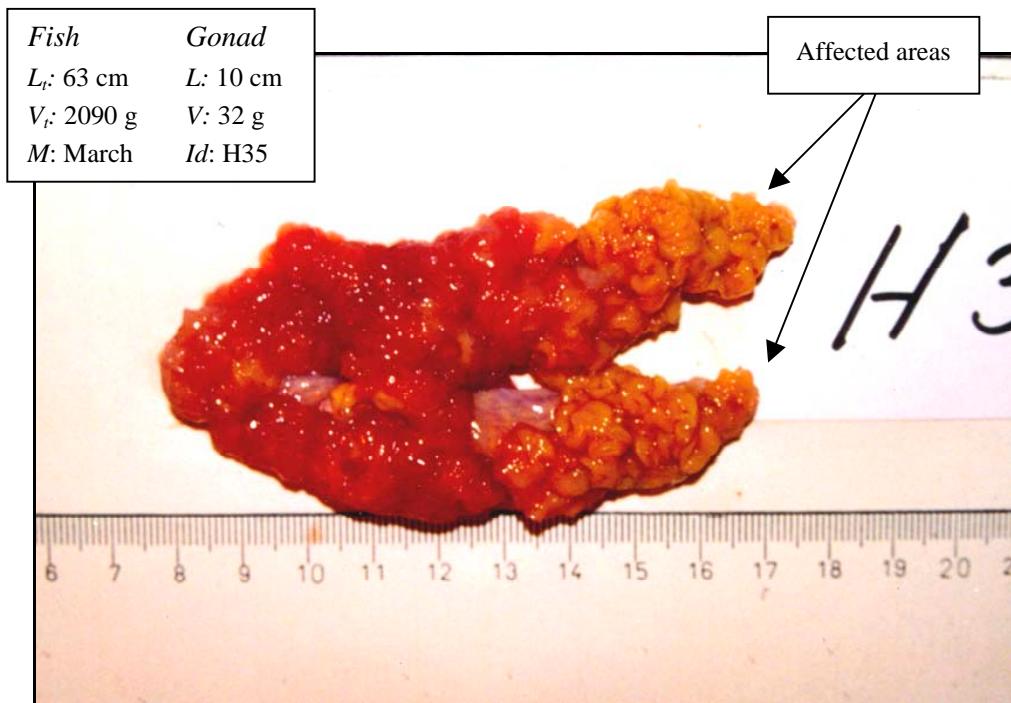
IX. Regeneration 2: Resting and spawning omission

(continued)



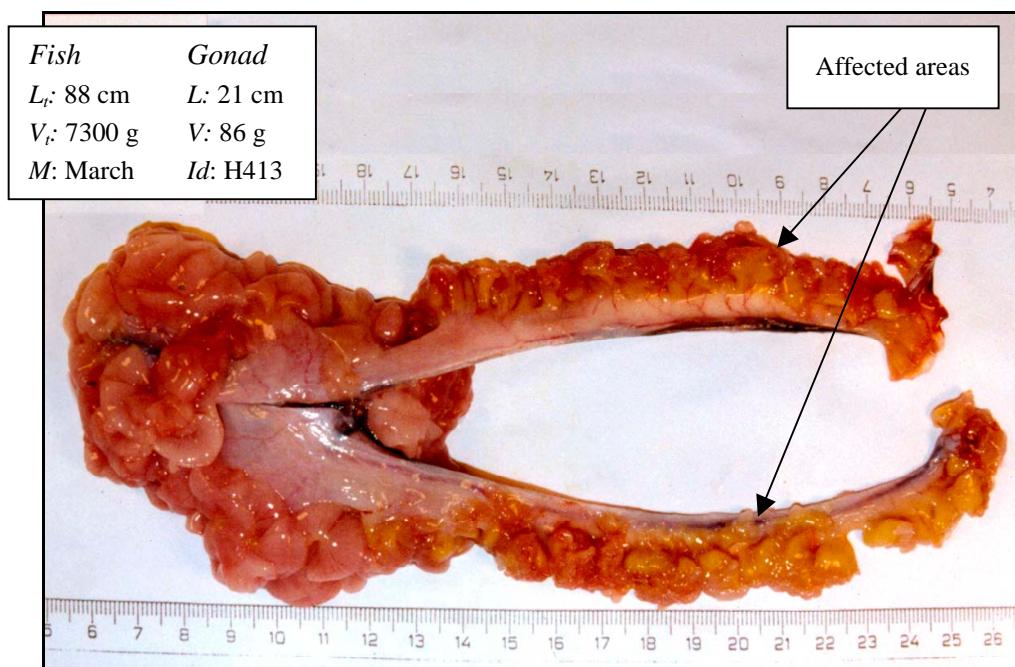
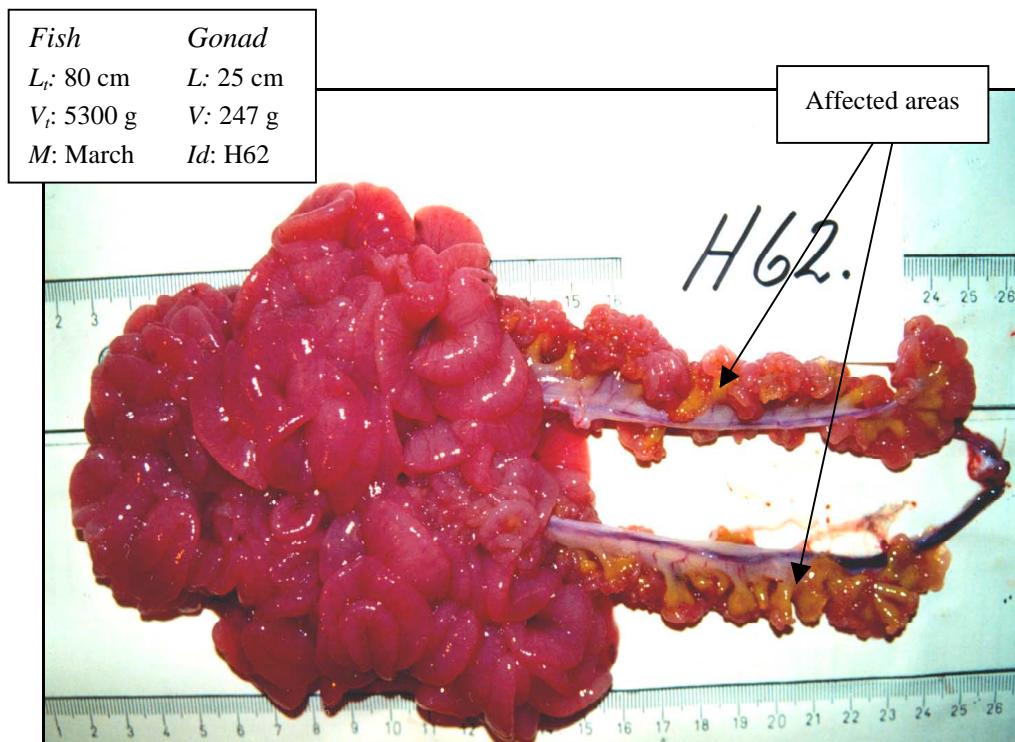
X. Degeneration: Reduced fertility

A: Testes with adipose tissue formation; affected parts undeveloped, hard, yellowish; non-affected parts with normal development. Observed in males from 50 cm. B: Other abnormalities.



X. Degeneration: Reduced fertility

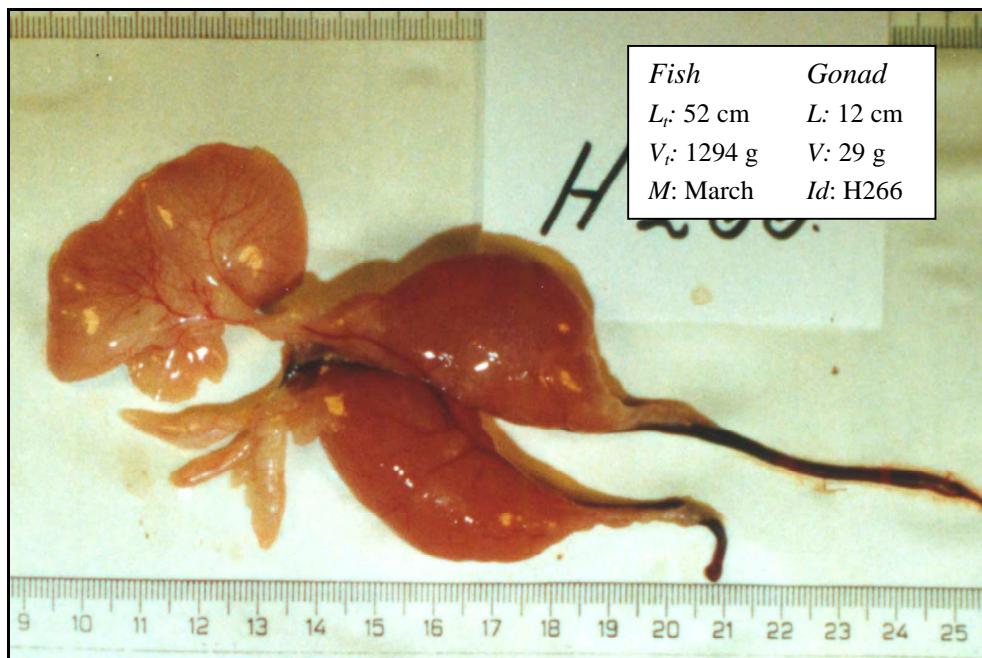
(continued)



X

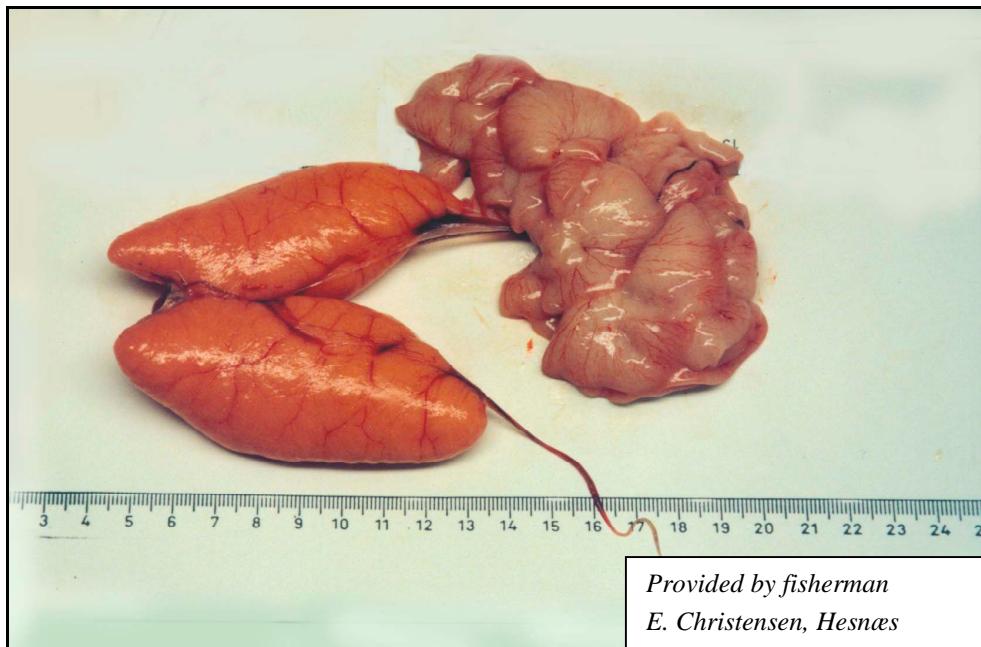
XX. Bisexuality

Gonad consisting of female and male components; transition between ovary and testes may be gradual or abrupt; in the first example the entire gonad is involved, while only one lobe of the gonad is affected in the other example.



XX. Bisexuality

(continued)



Provided by fisherman

E. Christensen, Hesnæs

(No data on fish)

M: January

Id: HX1

DFU-rapporter – index

Denne liste dækker rapporter udgivet i indeværende år samt de foregående to kalenderår. Hele listen kan ses på DFU's hjemmeside www.dfu.min.dk, hvor de fleste nyere rapporter også findes som PDF-filer.

- Nr. 74-00 Fisk, fiskeri og bundfauna ved Agerø, Limfjorden. Erik Hoffmann og Per Dolmer
- Nr. 75-00 Fisk og fiskebestande i Limfjorden 1984 – 1999. Erik Hoffmann
- Nr. 76-00 Genudlægninger af små blåmuslinger (*Mytilus edulis* L.) på vækstbanker i Limfjorden, 1999. Per Sand Kristensen, Nina Holm og Alex Hansen
- Nr. 77-00 A check list for multi-instrument projects. Harald Martens og Charlotte Jacobsen
- Nr. 78-00 Udvikling af standard garnserie til brug ved bestandsanalyse af flad- og rundfisk i marine lavvandede områder. Ole Ritzau Eigaard, Josianne Støttrup og Holger Hovgård
- Nr. 79-00 Undersøgelse af eventuelle miljøpåvirkninger ved anvendelse af hjælpestoffer og medicin i ferskvandsdambrug samt metoder til at reducere/eliminere sådanne påvirkninger. Samarbejdsprojekt mellem Danmarks Miljøundersøgelser (Redaktør), Danmarks Fiskeriundersøgelser, Kongelige Veterinære og Landbohøjskole og Dansk Dambrugerforening. (*udsolgt*)
- Nr. 80-00 Laks og havørreds gydevandring i Gudenåen i 1994 og 1995. Kim Aarestrup og Niels Jepsen
- Nr. 81-00 Hjertemuslinger (*Cerastoderma edule*) på fiskebanerne omkring Grådyb i Vadehavet, 2000. Per Sand Kristensen
- Nr. 82-00 Danmarks Fiskeriundersøgelser's Ramme- og aktivitetsplan 2000-2003. Danmarks Fiskeriundersøgelser
- Nr. 83-00 Dansk Laksefiskeri i Østersøen 1998/1999. Frank I. Hansen
- Nr. 84-00 Indsatsprojekt rapport 3. Fiskeriindsats og fiskeridødelighed, Østersøen. J. Rasmus Nielsen
- Nr. 85-00 Indsatsprojekt rapport 5. Fiskeriindsats og fiskeridødelighed, industrifiskeri. Paul Marchal, J. Rasmus Nielsen og Holger Hovgård (*udsolgt*)
- Nr. 86-00 Indsatsprojekt rapport 4. Fiskeriindsats og fiskeridødelighed, Kattegat. Holger Hovgård, , J. Rasmus Nielsen og Paul Marchal
- Nr. 87-01 Blåmuslingebestanden i det danske Vadehav efteråret 2000. Per Sand Kristensen og Niels Jørgen Pihl
- Nr. 88-01 Genudlægninger af blåmuslinger (*Mytilus edulis* L.) på vækstbanker i Limfjorden, 2000. Per Sand Kristensen og Nina Holm

- Nr. 89-01 Indsatsprojekt rapport 7. Fiskernes holdning til og accept af fiskeriregulering. Jesper Raakjær Nielsen og Christoph Mathiesen (*udsolgt*)
- Nr. 90-01 Hesterejer (*Crangon crangon*) – køns- og størrelsesfordelinger i danske fangster og landinger fra Nordsøen, 2000. Per Sand Kristensen og Agnethe Hedegaard
- Nr. 91-01 Danmarks Fiskeriundersøgelser's Ramme- og aktivitetsplan 2001-2004. Danmarks Fiskeriundersøgelser
- Nr. 92-01 Blåmuslinger (*Mytilus edulis* L.) i det nordlige Bælthav i 1996 (fiskerizone 30, 31 og 34). Forekomster og fiskeri. Per Sand Kristensen
- Nr. 93-01 Udsætningsforsøg med 18-28 cm ørred (*Salmo trutta* L.) i vandløb 1995-1998. Stig Pedersen og Peter Geertz-Hansen
- Nr. 94-01 Simulation model for evaluation of effert and catch quota management regimes. Per J. Sparre
- Nr. 95-01 Fiskebestande og fiskeri 2002. Sten Munch-Petersen.
- Nr. 96-02 Genudlægninger af blåmuslinger (*Mytilus edulis* L.) på vækstbanker i Limfjorden 2001. Per Sand Kristensen og Nina Holm.
- Nr. 97-02 Indsamling af detaljerede oplysninger om tobisfiskeriet i Nordsøen. Februar 2002. Henrik Jensen, Henrik Mosegaard, Anna Rindorf, Jørgen Dalskov og Palle Brogaard
- Nr. 98-02 Danmarks Fiskeriundersøgelser. Ramme- og Aktivitetsplan 2002-2005. Danmarks Fiskeriundersøgelser
- Nr. 99-02 Skjern Å's lampretter. Statusrapport fra naturovervågningen før restaureringen. Nicolai Ørskov Olsen, Hans-Christian Ingerslev, Henrik Dam og Christian Dieperink. (*udsolgt*)
- Nr. 100-02 Fangster af laksefisk fra Skjern Å og Storåen. Christian Dieperink.
- Nr. 101-02 Blåmuslinger (*Mytilus edulis* L.) i Lillebælt i 1995 (fiskerizone 40 - 44). Forekomster og fiskeri. Per Sand Kristensen
- Nr. 102-02 Hesterejer (*Crangon crangon*) – køns - og størrelsesfordelinger i danske fangster og landinger fra Nordsøen, 2001. Per Sand Kristensen og Agnethe Hedegaard
- Nr. 103-02 Dansk laksefiskeri i Østersøen 2001 og Status for forsøg med forsinket udsatte laks ved Bornholm og Møn. Frank Ivan Hansen og Stig Pedersen
- Nr. 104-02 Forbrugernes kvalitetsopfattelse af frossen fisk. Baseret på to fokusgrupper. Francisca Listov-Saabye
- Nr. 105-02 Forbrugerundersøgelse af frossen og optøet torsk. Francisca Listov-Saabye

- Nr. 106-02 Udredning vedrørende vandforbrug ved produktion af regnbueørreder i danske dambrug. Alfred Jokumsen. Rapporten er udarbejdet for Skov- og Naturstyrelsen (*udsolgt*)
- Nr. 107-02 Torskeopdræt – forskningsresultater og kundskab om torskeopdræt. Josianne G. Støttrup
- Nr. 108-02 Hjertemuslinger (*Cerastoderma edule*) på fiskebanerne omkring Grådyb i Vadehavet, 2002. Per Sand Kristensen, Niels Jørgen Pihl og Alex Hansen
- Nr. 109-02 Delrapport vedr. klimaændringer. Udvalget om Miljøpåvirkninger og Fiskeriressourcer. Brian R. MacKenzie, André W. Visser, Jes Fenger, Poul Holm
- Nr. 110-02 Delrapport vedr. eutrofiering. Udvalget om Miljøpåvirkninger og Fiskeriressourcer. Helge Thomsen, Torkel G. Nielsen, Katherine Richardson
- Nr. 111-02 Delrapport vedr. miljøfremmede stoffer. Udvalget om Miljøpåvirkninger og Fiskeriressourcer. Stig Møllergaard, Britta Pedersen, Valery Forbes, Bente Fabeck, Alf Aagaard
- Nr. 112-02 Delrapport vedr. habitatpåvirkninger. Udvalget om Miljøpåvirkninger og Fiskeriressourcer. Per Dolmer, Karsten Dahl, Søren Frederiksen, Ulrik Berggren, Stig Prüssing, Josianne Støttrup, Bo Lundgren
- Nr. 113-02 Delrapport vedr. topprædatorer. Udvalget om Miljøpåvirkninger og Fiskeriressourcer. Erik Hoffmann, Christina Lockyer, Finn Larsen, Palle Udh Jepsen, Thomas Bregnbae, Jonas Teilmann, Lene J. Scheel-Bech, Ellen Stie Kongsted, Henning Thøgersen
- Nr. 114-02 Delrapport vedr. andre faktorer. Udvalget om Miljøpåvirkninger og Fiskeriressourcer. Stig Møllergaard, Per Dolmer, Ulrik Berggren, Torben Wallach
- Nr. 115-02 Fiskebestande og fiskeri i 2003. Sten Munch-Petersen.
- Nr. 116-02 Manual to determine gonadal maturity of Baltic cod. Jonna Tomkiewicz, Lars Tybjerg, Nina Holm, Alex Hansen, Carl Broberg, Erik Hansen

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