## Title page

- 1. Title
- : Sex Characterization of wrasses inhabiting in the coastal waters of Jeju, Korea
- : 제주연안에 서식하는 놀래기류의 성특성
- 2. Author's name
- : Chi Hoon Lee, Yoon Seok  $\text{Kim}^1$  and Young Don  $\text{Lee}^\dagger$
- : 이치훈・김윤석<sup>1</sup>・이영돈<sup>†</sup>
- 3. Address
- : Marine and Environmental ResearchInstitute, Cheju National University, Jeju 690-968, Korea
- : <sup>1</sup> Korean Minjok Leadership Academy, Gangwon 225-823, Korea
- : 제주대학교 해양과환경연구소, <sup>1</sup>민족사관고등학교
- 4. Running title
- : Sex Characterization of wrasses
- 5. Correspondence Author
- : Young-Don Lee
- : Marine and Environmental Research Institute, Cheju National University,

3288Hamdoek, Jocheon, Jeju 690-968, Korea.

Tel/Fax: 82-64-782-8922, e-mail: leemri@cheju.ac.kr

# Sex Characterization of Wrasses Inhabiting in the Coastal Waters of Jeju, Korea

Chi Hoon Lee, Yoon Seok Kim<sup>1</sup> and Young Don Lee<sup>†</sup>

Marine and Environmental Research Institute, Cheju National University, Jeju 690-968, Korea <sup>1</sup> Korean Minjok Leadership Academy, Gangwon 225-823, Korea

제주연안에 서식하는 놀래기류의 성특성

이치훈·김윤석<sup>1</sup>·이영돈<sup>†</sup> 제주대학교 해양과환경연구소, <sup>1</sup>민족사관고등학교

ABSTRACT: We reviewed sex-change patterns in the wrasses *Halichoeres poecilopterus*, *H. tenuispinis*, *Pteragogus flagellifer*, and *Pseudolabrus sieboldi* inhabiting the coastal waters of Jeju, Korea, based on the sex distribution according to standard length and sex characteristics of the gonads. *Halichoeres poecilopterus*, *H. tenuispinis*, *Pt. flagellifer*, and *Ps. sieboldi* are protogynous hermaphroditic fish. Histological observations revealed that these wrasses are undelimited type 2 species because testicular tissue appears in most parts of the gonads during ovary degeneration. Both initial- and terminal-phase males were present in the investigated populations, indicating that *Halichoeres poecilopterus*, *H. tenuispinis*, and *Ps. sieboldi* are of the diandric type. In contrast, *Pt. flagellifer* is considered a monandric type, because all males in the investigated populations were terminal-phase males produced via sex change from functional females.

Key words: Wrasse, Sex change, Protogynous hermaphroditic fish, Undelimited type, Monandry type

**요 약**: 제주 연안에 서식하는 용치놀래기, 어렝놀래기, 황놀래기, 놀래기를 대상으로 체장에 따른 성분포와 생식소의 성특성을 조직학적으로 탐색하여 성전환 양상을 비교하였다. 용치놀래기, 어렝 놀래기, 황놀래기, 놀래기는 자성선숙형 자웅동체어로 조직학적 관찰결과 정소조직이 난소의 퇴화 중에 생식소 전역에 출현하는 혼재형 2에 속하는 어종이었다. 용치놀래기, 황놀래기 그리고 놀래 기는 1차 수컷과 2차 수컷이 존재하는 복웅성 어류이고 조사된 어렝놀래기는 암컷의 일부가 수컷 으로 성전환하여 2차 수컷만이 나타나고 있어 단웅성 어류라고 생각된다.

#### Introduction

The sex of fish can be divided into gonochorism and hermaphrodism. Hermaphrodites can be divided into protogynous, protandrous, and synchronous types. Protogynous hermaphrodites first become sexually mature as females and later change sex to become males. Protandrous hermaphrodites first become sexually mature as males and then later change sex to become females. Synchronous hermaphrodites simultaneously function as males and females (Atz, 1964; Yamamoto, 1969). The diversity of fish sexes is a reproductive strategy for survival in marine environments.

Wrasses are protogynous hermaphroditic fish. Many studies have investigated their reproductive biology, including sex change and sexual patterns (Bruslé, 1987 Warner and Robertson, 1987), age and growth (Hashimoto et al., 1991), spawning behavior (Sakai and Kohda, 2001), and reproductive cycle ((Lee et al., 1991, 1992a, b, 1993 Candi et al., 2004). About 10 species of wrasse inhabit the waters off Jeju Island, Korea. Here, we review the sex change patterns of *Halichoeres poecilopterus, H. tenuispinis, Pteragogus flagellifer*, and *Pseudolabrus sieboldi* inhabiting the coastal waters off Jeju Island, Korea, based on the sex distribution according to standard length and sex characteristics of the gonads.

### Gonadal changes during sex change

The process of sex change in the wrasse *Thalassoma duperrey* is divided into six stages based on gonadal changes (Nakamura et al., 1989): Stage 1, normal ovaries of females arefilled with vitellogenic oocytes during the breeding season; Stage 2, onset of sex change, during which the degeneration of yolky oocytes occurs; Stage 3, degeneration of peri-nucleolus oocytes; Stage 4, proliferation of Leydig cells and spermatogonia; Stage 5, onset of spermatogenesis; Stage 6, completion of testes just after sex change.

The ovaries of *T. duperrey* are filled with vitellogenic oocytes during the breeding season, but contain no spermatogenic tissue (Nakamura et al., 1989). *Thalassoma duperrey* is considered a protogynous hermaphrodite, as are *Anthias squamipinnis* (Shapiro, 1981), *H. poecilopterus* (Lee et al., 1991), *H. tenuispinis* (Lee et al., 1993), *Pt. flagellifer*(Lee et al., 1992a), and *Ps. sieboldi* (Lee et al., 1992b).

The testes of *Ps. sieboldi* primary males consist of numerous testicular lobules, and efferent sperm ducts are located at the base of the lobules (Fig. 1A). The ovary is arranged in lamellae, which extend into a central, membrane-bound ovarian cavity. The lamellae contain young oocytes and primary yolk oocytes (Fig. 1B). The gonads of sex-changing individuals consist of testicular tissue at the cortex and ovarian tissue at the medulla (Fig. 1C). A few degenerating yolk oocytes still remain in the spermatogenic gonads (Fig. 1D). The testes of terminal males following the sex change from female to male contain an ovarian cavity, and newly formed efferent sperm ducts are situated along the base of the gonads (Fig. 1E).

In many families and species, including certain labrids such as T. duperrey, scarids, and several synbranchids, primary and terminal males can be distinguished histologically because of distinct morphological differences between the two testis types (Liem, 1968 Harrington, 1971; Nakamura et al., 1989). However, this morphological distinction may not be as clear in all labrids (Shapiro and Rasotto, 1993). Protogynous hermaphroditic fish can be divided into two types, delimited and undelimited, according to the location of the testicular tissue during sex change (Sadovy and Shapiro, 1987). In the delimited type, the origin of testicular tissue is adjacent to ovarian tissue, and both testicular and ovarian tissue are separated by connective tissue. This type includes species of the Sparidae (D'Ancona, 1949), (Reinboth, 1962), and Centropyge interruptus of the Pomacanthidae (Moyer and Nakazono, 1978). In the undelimited type, oogenesis and spermatogenesis occur simultaneously in several parts of the gonads, andovarian and testicular tissue are not separated by connective tissue. The undelimited type can be divided into two types: undelimited type 1, in which testicular tissue appears in a particular area, and undelimited type 2, in which testicular tissue appears in most parts of the gonads during ovary degeneration. Undelimited type 1 includes Rypticus of the Serranidae (Smith, 1965) and Coryphopterus of the Gobiidae (Cole, 1983). Undelimited type 2 includes Genicanthus melanospilos of the Pomacanthidae (Shen and Liu, 1976), Labridae (Roede, 1972; Reinboth, 1975), and Scaridae (Choat and Robertson, 1975; Bruce, 1980). Halichoeres poecilopterus (Lee et al., 1991), H. tenuispinis(Lee et al., 1993), Pt. flagellifer (Lee et al., 1992a), and Ps. sieboldi (Lee et al., 1992b) inhabiting the coastal

Sex steroid hormones play a critical role in the sex change of hermaphroditic fish. The predominant steroid associated with sex change in protandrous species is estradiol-17 $\beta$  (E<sub>2</sub>). In contrast, 11-ketotestosterone (11-KT) plays a critical role in protogynous species (Chang and Lin, 1998; Kroon and Liley, 2000). For example, during sex change in the wrasse *T. duperrey*, plasma levels of E<sub>2</sub> decrease, whereas those of 11-KT increase (Nakamura et al., 1989). However, during sex change in the protogynous *Monopterus albus*, the development of interstitial Leydig cells precedes the increase in 11-KT production, and the increase in the number of Leydig cells is accompanied by an increase in 11-KT production (Chan and Phillips, 1967). Therefore, because interstitial cells such as Leydig cells are accompanied by endocrine activity according to sex change (Tang et al., 1974, 1975), the endocrine activity of Leydig cells is considered a factor in sex change (Chan and Yeung, 1983).

waters off Jeju Island are considered undelimited type 2 species.

The degeneration of oocytes and proliferation of somatic and acidophillic interstitial cells have been observed during sex change in *H. poecilopterus* (Lee et al., 1991), *H. tenuispinis* (Lee et al., 1993), *Pt. flagellifer* (Lee et a., 1992a), and *Ps. sieboldi* (Lee et al., 1992b). This is considered evidence that the endocrine activity of interstitial cells promotes sex change.

#### Sex distribution according to standard length

The sex distribution according to the standard length of *H. poecilopterus* (Lee et a., 1991), *H. tenuispinis*(Lee et al., 1993), *Pt. flagellifer* (Lee et a., 1992a), and *Ps. sieboldi* (Lee et al., 1992b) is shown in Fig. 2. In *H. poecilopterus*, females (n = 41) were 10.0–17.0 cm in standard length (SL), initial males (n = 11) were 10.5–16.5 cm SL, inter-sex (n = 6) individuals were 11.0–16.0 cm SL, and terminal females (n = 23) were 11.0–18.0 cm SL (Fig. 2A). In *Pt. flagellifer*, females (n = 26) were 8.0–12.5 cm SL, inter-sex fish (n = 16) were 9.5–15.0 cm SL, and terminal females (n = 75) were 11.5–18.0 cm SL (Fig. 2B). In *Ps. sieboldi*, females (n = 56) were 9.0–17.0 cm SL, initial males (n = 75) were 13.5–16.5 cm SL, inter-sex individuals (n = 9) were 11.5–16.0 cm SL, and terminal females (n = 75) were 11.5–18.0 cm SL (Fig. 2C). In *H. tenuispinis*, females (n = 73) were 8.5–13.5 cm SL, inter-sex individuals (n = 2) were 11.0–13.0 cm SL, and terminal females (n = 14) were 10.0–13.0 cm SL (Fig. 2D).

#### Sex pattern of wrasses

Sex change in fish is species specific and has been reported in at least 23 families, including over 350 species (Helfman et al., 1997). Protogynous hermaphroditic species are known from the Gobiidae (Cole, 1990; Cole and Shapiro, 1992), Labridae (Nakazono and Kusen, 1991; Lee et al., 1993), Scaridae (Kusen and Nakazono, 1991), and Serranidae (Tanaka et al., 1990; Lee et al., 1993). Protandrous hermaphrodites include species from the Pomacentridae (Moyer and Nakaozono, 1978) and *Acanthopagrus schlegeli* of the Sparidae (Chang and Lin, 1998).

Protogynous hermaphroditic fish can be divided into two types, monandric and diandric, on the male developmental pathway (Reinboth, 1967). Monandric species follow a singlemale developmental pathway; all males in a population are terminal males derived exclusively from functional females via sex change. Species of this type include *Nelabrichthys ornatus* (Andrew et al., 1996), *Achoerodus viridis* (Gillanders, 1995), *Cirrhilabrus temmincki* (Kobayashi and Suzuki, 1990), *Choerodon schoenleinii* (Ebisawa et al., 1995), *C. azurio* (Nakazono and Kusen 1991), and *Calotomus japonicus* of the Scaridae (Kusen and Nakazono, 1991). Diandric species follow two male developmental pathways, i.e., initial males develop from juveniles through sexual differentiation and terminal males develop via sex change as in monandric species; both types occur within a population. Species of this type include *Thalassoma bifasciatum* (Warner and Robertson, 1978), *Cheilinus undulatus* (Donaldson and Sadovy, 2001), and scarids (Robertson and Warner, 1978).

Halichoeres poecilopterus (Lee et a., 1991), H. tenuispinis (Lee et al., 1993), and Ps. sieboldi (Lee et al., 1992b) are of the diandric type because both initial- and terminal-phase males were present in the investigated populations. However, Pt. flagellifer (Lee et al., 1992a) a monandric type because all males

in the investigated population were terminal-phase males resulting from sex change from functional females. Based on the gonads and sex distribution according to standard length, *H. poecilopterus*, *H. tenuispinis*, and *Ps. sieboldi* had differentiated testes and ovaries during sex differentiation; some female *H. poecilopterus* and *H. tenuispinis* changed into males after maturation, whereas most female *Ps. sieboldi* became males after maturation. However, *Pt. flagellifer*had differentiated ovaries, and all individuals changed into males after maturation (Fig. 3).

The species-specific sex change of monandric and diandric fish is considered a reproductive strategy for surviving in the marine environment. However, detailed studies are needed to further understand which type is more advantageous in species maintenance, as well as to determine the difference between females that can change sex and females that do not have the genetic capacity to change sex in diandric fish.



#### REFERENCES

- Andrew TG, Buxton CD, Hecht T (1996) Aspects of the reproductive biology of the concha wrasse, *Nelabrichthys ornatus*, at Tristan da Cunha. Environ Biol Fish 46: 139-149.
- Atz JW (1964) Intersexuality in fishes. In: Armstrong CN & Marshall AJ (ed.), Intersexuality in Vertebrates Including Man. Academic Press, London, pp 145-232.
- Bruce RW (1980) On the occurrence of very small terminal phase parrotfish. Copeia 1980: 887-889.
- BrusléS (1987) Sex-inversion of the hermaphroditic, protogynous teleost Coris julis L. (Labridae). J Fish Biol 30: 605-616.
- Candi G, Castriota L, Andaloro F, Finoia MG, Marino G (2004) Reproductive cycle and sex reversion in razor fish, a protogynous labrid in the southern Mediterranean Sea. J Fish Biol 64: 1498-1513.
- Chan STH, Phillips JG (1967) The structure of the gonad during natural sex reveral in *Monopterus albus*. J Zool (Lond.) 153: 129-141.
- Chan STH, Yeung WSB (1983) Sex control and sex reversal in fish under natural conditions. In: Hoar WS, Randall DJ, Donaldson EM (ed.), Fish Physiology. Academic Press, New York, pp 171-222.
- Chang CF, Lin BY (1998) Estradiol-17βstimulates aromatase activity and reversible sex change in protandrous black porgy, *Acanthopagrus schlegeli*. J Exp Zool 280: 165-173.
- Choat JH, Robertson DR (1975) Protogynous hermaphroditism in fishes of the family Scaridae. In: Reinboth R (ed.), Intersexuality in the Animal Kingdon. Springer-Verlag, Berlin, pp 263-283.
- Cole KS (1983) Protogynous hermaphroditism in a temperatezone territorial marine goby, *Coryphopterus nicholsi*. Copeia 1983: 809-812.
- Cole KS (1990) Patterns of gonad structure in hermaphroditic gobies Teleostei Gobiidae. Environ Biol Fishe 28: 125-142.
- Cole KS, Shapiro DY (1992) Gonadal structure and populationcharacteristics of the protogynous goby *Coryphopterus glaucofraenum*. Mar Biol 113: 1-9.
- D'Ancona U (1949) Ermafroditism ed intersessualita nei Teleostei. Experientia 5: 381-389.
- Donaldson TJ and Sadovy Y (2001) Threatened fishes of the world: *Cheilinus undulatus* Rüppell, 1835 (Labridae). Environ Biol Fish 62: 428.
- Ebisawa A, Kanashiro K, Kyan T, Motonaga F (1995) Aspects of reproduction and sexuality in the back-spot tuskfish, *Choerodon schoenleinii*. Japan J Ichthyol 42: 121-130.
- Gillanders BM (1995) Reproductive biology of the protogynous hermaphrodite *Achoerodus viridis* (Labridae) from south-eastern Australia. Mar and Freshwat Res 46: 999-1008.
- Harrington RWJr (1971) How ecological and genetic factors interact to determine when self-fertilizaing hermaphrodities of *Rivulus marmoratus*change into functional secondary males

with a reappraisal of the modes of intersexuality among fishes. Copeia 1971: 389-432.

- Hashimoto H, Gushima K, Kakuda H (1991) On the age and growth of the labroid fish *Halichoeres poecilopterus* from the Seto Naikai, Japan Nippon Suisan Gakkaishi 57:1457-1462.
- Helfman GS, Collette, BB, Facey DE (1997) The Diversity of Fishes. Blackwell Science, Malden, MA, 544 pp.
- Kobayasi K, Suzuki K (1990) Gonadogenesis and sex succession in the protogynous wrasse, *Cirrhilabrus temmincki*, in Suruga Bay, Central Japan. Japan J Ichthyol 37: 256-264.
- Kroon FJ, Liley NR (2000) The role of steroid hormones in protogynous sex change in the blackeye goby, *Coryphopterus nicholisii* (Teleostei: Gobiidae). Gen Comp Endocrinol 118: 273-283.
- Kusen JD, Nakazono A (1991) Protogynous hermaphroditism in the parrotfish, *Calotomus japonicus*. Jpn J Ichthyol 38: 41-46.
- Lee YD, An CM, Lee JJ, Lee TY (1992b) Reproductive cycle and sex reversal of *Pseudolabrus japonicus* (Houttuyn). Bull Mar Res Inst Cheju Natl Univ 16: 55-66.
- Lee YD, Go HB, Kim HB, Park IS, Lee JJ (1993) Sex reversal of protogynous hermaphrodite fish. Bull Mar Res Inst Cheju Natl Univ 17: 115-127.
- Lee YD, Go YB, Chung SC (1992a) Reproductive cycle and sex reversal of the cock-tail wrasse, *Pteragogus flagellifer*. Bull Mar Res Inst Cheju Natl Univ 16: 43-53.
- Lee YD, Rho HK, Lee TY (1991) Reproductive ecology of the wrasse, *Halichoeres poecilopterus* (Temmink et Schlegel). Bull Mar Res Inst Cheju Natl Univ 15: 93-102.
- Liem KF (1968) Geographical and taxonomic variation the pattern of natural sex reversal in the teleost fish order Synbranchiformes. J Zool (Lond.) 156: 225-238.
- Moyer J, Nakazono A (1978) Protandrous hermaphroditism in sex species of the anemonefish genus *Amphiprion* in Japan. Jpn J Ichthyol 25: 101-106.
- Moyer JT, Nakazono A (1978) Population structure, reproductive behavior and protogynous hermaphroditism in the angelfish *Centropyge interruptus* at Miyake-jima, Japan. Ibid 25: 25-39.
- Nakamura M, Hourigan TF, Yamauchi K, Nagahama Y, Grau EG (1989) Histological and ultrastructural evidence for role of gonadal steroid hormones in sex change in the protogynous wrasse Thalassoma duperrey. Env Biol Fish 24: 117-136.
- Nakazono A ,Kusen JD (1991) Protogynous hermaphroditism in the wrasse *Choerodon azurio*. Nippon Suisan Gakkaishi 57: 417-420.
- Reinboth R (1962) Morphologische und funktionelle Zweigeschlichkeit bei marinen Teleostiern (Serranidae, Sparidae, Centracanthidae, Labridae). Zool Jb (Physiol.) 69: 405-480.
- Reinboth R (1967) Biandric teleost species. Gen Comp Endocrinol 9: 486.
- Reinboth R (1975) Spontaneous and hormone-induced sex inversion in wrasse (Labridae). Pubbl

Staz Zool Napoli 39 (Suppl.) : 550-573.

- Robertson DR, Warner RR (1978) Sexual patterns in the labroid fishes of the western Caribbean. 2. The parrotfish (Scaridae). Smithson Contrib Zool 225: 1-26.
- Roede MJ (1972) Color as related to size, sex, and behavior in seven Caribbeanlabrid fish species (genera Thalassoma, Halichoeres and Hemipteronotus). Stud Fauna Curacao 42: 1-266.
- Sadovy Y, Shapiro DY (1987) Criteria for the diagnosis of hermaphroditism in fishes. Copeia 1987: 136-156.
- Sakai Y, Kohda M (2001) Spawning timing of the cleaner wrasse, *Labroides dimidiatus*, on a warm temperate rocky shore. Ichthyol Res 48: 23-30.
- Shapiro DY (1981) Size, maturation and the social control of sex reversal in the coral reef fish Anthias squamipinnis. J Zool 193: 105-128.
- Shapiro DY, Rasotto MB (1993) Sex differentiation and gonadal development in the diandric, protogynous wrasse, Thalassoma bifasciatum (Pisces, Labridae). J Zool (Lond.) 230: 231-245.
- Shen SC, Liu CH (1976) Ecological and morphorogical study of the fish fauna from the waters around Taiwan and its adjacent islands. 17. A study of sex reversal in a pomacanthid fish, *Genicanthus semifasciatus* (Kamohara). Sci Rep Nat Taiwan Univ 6: 140-150.
- Smith CL (1965) The patterns of sexuality and the classification of serranid fishes. Amer Mus Novit 2207: 1-20.
- Tanaka H, Hirose K, Nogami K, Hattori K, Ishibashi N (1990) Sexual maturation and sex reversal in red spotted grouper, *Epinephelus akaara*. Bull Natl Res Inst Aquacult 17: 1-15.
- Tang F, Chan STH, Lofts B (1974) Effect of steroid hormones on the process of natural sex reveral in the rice-filed eel, *Monopterus ablus*. Gen Comp Endocrinol 24: 227-241.
- Tang F, Chan STH, Lofts B (1975) A study on the 3β-and 17β-hydroxysteroid dehydrogenase activities in the gonads of *Monopterus ablus* at various sexual phases during natural sex reversal. J Zool (Lond.) 175: 571-580.
- Warner RR and Robertson DR (1978) Sexual patterns in the labroid fishes of the western Caribbean. 1. The Wrasses (Labridae). Smithson Contrib Zool 254: 1-27.
- Yamamoto T (1969) Sex differentiation. In: Hoar WS & Randall DJ (ed.), Fish Physiology, Academic Press, New York, pp 117-175.

# Figure Legends

Fig. 1. Histological observations of gonadal structure in sex-changing wrasse. Ed, efferent duct; Pn, perinucleolar oocyte; Oc, ovarian cavity; Op, ovarian part; Py, primary yolk oocyte; St, seminiferous tubules; Tp, testicular part (Lee et al., 1993).

Fig. 2. Relationship between sex distribution and standard length. A, *Halichoeres poeciopterus* B, *Pteragogus flagellifer* C, *Pseudolabrus sieboldi* D, *Halichoeres tenuispinis* (Lee et al., 1993).

Fig. 3. The sex change pattern of the wrasses inhabiting in the coastal waters of Jeju, Korea



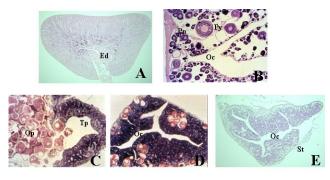
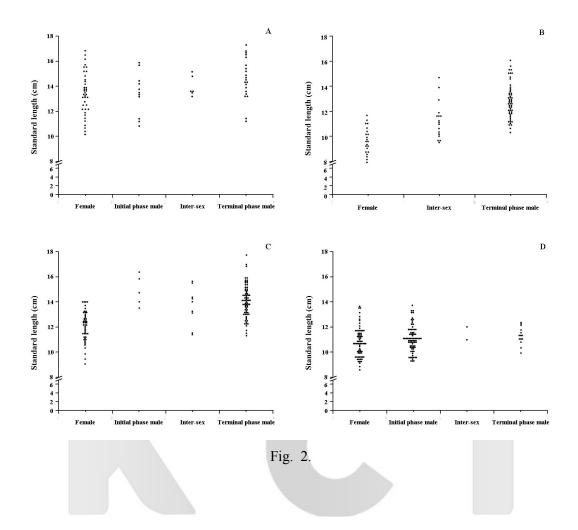


Fig. 1.





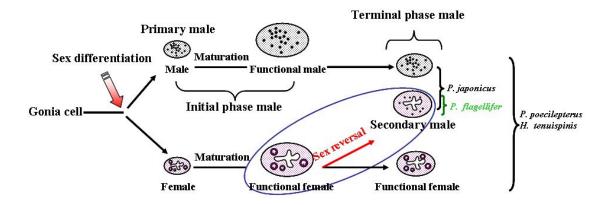


Fig. 3.

